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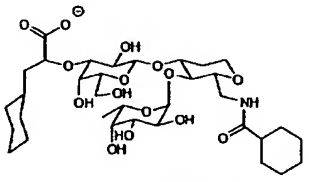
(54) Title: OLIGOSACCHARIDES AND CONJUGATES THEREOF FOR THE TREATEMENT OF PSEUDOMONAS BACTERIA INFECTION



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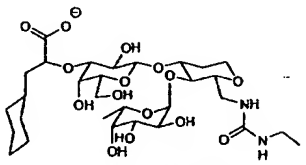

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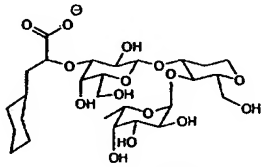

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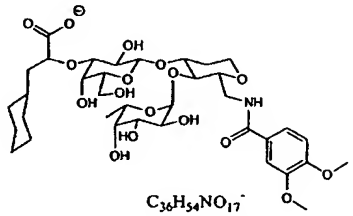

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(57) Abstract: Compositions and methods are provided related to Pseudomonas bacteria. The compositions and methods may be used for diagnosis and therapy of medical conditions involving infection with Pseudomonas bacteria. Such infections include Pseudomonas aeruginosa in the lungs of patients with cystic fibrosis. A compound useful in the present methods may be linked to a therapeutic agent. Pseudomonas bacteria may be inhibited by blocking colonization, arresting growth or killing the bacteria.

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OLIGOSACCHARIDES AND CONJUGATES THEREOF FOR THE TREATMENT OF PSEUDOMONAS BACTERIA INFECTION

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to compounds, compositions and methods for the diagnosis and therapy of diseases in warm-blooded animals (e.g., in humans) involving infections with and colonization by *Pseudomonas* bacteria, including *Pseudomonas aeruginosa* in the lungs of patients with cystic fibrosis. The invention relates more particularly to the use of one or more compounds selective for binding *Pseudomonas* bacteria. These compounds are useful for diagnosis and/or therapeutic intervention of the colonization of *Pseudomonas* bacteria, or may be linked to an agent(s) to target and effectively arrest or kill *Pseudomonas* bacteria.

Description of the Related Art

Pseudomonas infections occur in a variety of medical conditions and can be life threatening. *Pseudomonas* is an opportunistic bacterium. Examples of individuals at risk include cystic fibrosis patients and burn patients. Cystic fibrosis is described below as a representative example of a medical condition which can involve infection with *Pseudomonas* bacteria.

Cystic Fibrosis (CF) is the most common lethal genetic disease among the Caucasian population. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which acts as a chloride channel. The genetic mutations of CFTR which alter ion movements also affect the N-glycosylation of CFTR as well as other cell surface molecules. All of the exocrine glands of the patients are affected; however, the lungs are the primary site of morbidity and mortality. The general change in glycosylation results in an increase in Lewis fucosylation and a decrease in sialylation. The salivary and respiratory musins from CF patients also contain higher levels of Lewis type oligosaccharides including sialylated and sulfated Lewis x/a structures.

The major cause of morbidity and mortality in CF patients is chronic lung colonization by the bacterium, *Pseudomonas aeruginosa*, which results in pronounced lung infection with a robust neutrophilic inflammatory response leading to destruction of the lungs and death. Colonization by *P. aeruginosa* initiates by the

binding of fimbrial and flagellar lectins on the bacteria to Lewis-type carbohydrate structures on the lung cell surfaces. These lectins, known as PA-IL and PA-IIL, bind these oligosaccharide structures with high affinity and represent a potential molecular target to block the first step of bacterial colonization. Patients that are never fully colonized by the bacteria maintain an excellent long-term prognosis. Due to the difficulties in the current approaches in the art for prevention of colonization in an individual by *Pseudomonas* bacteria, there is a need for improved compounds, compositions and methods.

BRIEF SUMMARY OF THE INVENTION

Briefly stated, this invention provides compounds, compositions and methods for utilizing lectins expressed on *Pseudomonas* bacteria for the detection of *Pseudomonas* bacteria and the diagnosis and therapy of disease involving *Pseudomonas* bacteria, including human disease. For example, glycomimetics of the Lewis structures that have high affinity binding to the lectins on *P. aeruginosa* will have a beneficial therapeutic effect on CF patients. Furthermore, these glycomimetics may be conjugated, for example, with strong antibiotics to increase the efficacy and lower the dose, thereby avoiding well known deleterious side effects of these potent antibiotics. Given that these binding sites are crucial for the colonization and pathogenicity of the bacterium, mutations in this target to become resistant to this conjugate therapy should result in non-pathogenic forms of the bacteria.

One embodiment of the present invention provides a method of inhibiting *Pseudomonas* bacteria in a warm-blooded animal comprising administering to the animal in an amount effective to inhibit the bacteria a compound comprising a compound according to Figure 1 or Figure 2.

In another embodiment, the present invention provides a conjugate comprising a therapeutic agent linked to a compound according to Figure 1 or Figure 2.

In another embodiment, the present invention provides a method of detecting *Pseudomonas* bacteria comprising contacting a sample with a diagnostic agent linked to a compound comprising a compound according to Figure 1 or Figure 2, under conditions sufficient for the compound to bind to the bacteria if present in the sample; and detecting the agent present in the sample, wherein the presence of agent in the sample is indicative of the presence of *Pseudomonas* bacteria.

In another embodiment, the present invention provides a method of immobilizing *Pseudomonas* bacteria on a solid support comprising contacting, under conditions sufficient for binding, a sample containing *Pseudomonas* bacteria with a

compound comprising a compound according to Figure 1 or Figure 2 that is immobilized on a solid support; and separating the sample from the solid support.

In other embodiments, the compounds and conjugates described herein may be used in the preparation of a medicament for the inhibition of *Pseudomonas* bacteria.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E show the structures of glycomimetic compounds.

Figures 2A-2B show the structures of additional glycomimetic compounds.

Figure 3 depicts the synthesis of representative glycomimetic compound 3.

Figure 4 depicts the synthesis of glycomimetic compound 21.

Figure 5 depicts the synthesis of representative glycomimetic compound 15.

Figure 6 depicts the acylation of intermediate 21 to give a variety of representative glycomimetic compounds.

Figure 7 depicts the synthesis of compound 22.

Figures 8A-8B depict the synthesis of compounds 4, 6, 7, 8, 10, 12, 13, 14, 16, 17, 18 and 19.

Figure 9 depicts the synthesis of compounds 22 and 23.

Figure 10 depicts the synthesis of intermediates.

Figure 11 depicts the synthesis of intermediates and compound 24.

Figure 12 depicts the synthesis of intermediates xxxxiv and xxxxv.

Figure 13 depicts the synthesis of compound 25.

Figure 14 depicts the synthesis of compound 26.

Figure 15 depicts the synthesis of intermediates xxxxxi and xxxxxii.

Figure 16 depicts the synthesis of compound 27.

Figure 17 depicts the synthesis of compound 28.

Figure 18 depicts the binding of PA-IIL lectin to immobilized neutral carbohydrate structures.

Figure 19 depicts the binding of PA-IIL lectin to immobilized acidic carbohydrate structures.

Figure 20 depicts the determination of assay conditions for IC_{50} values of PA-IIL lectin inhibition.

Figure 21 shows a schematic representation of the assay developed for determining IC_{50} values of glycomimetic inhibitors of PA-IIL lectin.

Figure 22 depicts the inhibition of PA-IIL lectin by glycomimetic compound 23.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides compounds and compositions that bind *P. aeruginosa* and may be used in the diagnosis and therapy of disease.

GLYCOMIMETIC COMPOUNDS

The term "Glycomimetic compound," as used herein, refers to a molecule that binds specifically to *P. aeruginosa*. The structures of the Glycomimetic compounds covered by this invention are shown in Figures 1 and 2, and also include the compounds disclosed herein except that the compounds do not contain the mimic for sialic acid which is shown as the terminal cyclohexyl lactic acid moiety. This is accomplished, for example, by eliminating the step involving the addition of intermediate E in certain of the reaction schemes. All compounds (or conjugates thereof) useful in the present invention include physiologically acceptable salts thereof.

For certain embodiments, it may be beneficial to also, or alternatively, link a diagnostic or therapeutic agent, such as a drug to a Glycomimetic compound, to form a conjugate where the linkage is covalent. As used herein, the term "therapeutic agent" refers to any bioactive agent intended for administration to a warm-blooded animal (e.g., a mammal such as a human) to prevent or treat a disease or other undesirable condition or to enhance the success of therapies. Therapeutic agents include antibiotics, hormones, growth factors, proteins, peptides, genes, non-viral vectors and other compounds.

GLYCOMIMETIC COMPOUND FORMULATIONS

Glycomimetic compounds as described herein may be present within a pharmaceutical composition. A pharmaceutical composition comprises one or more Glycomimetic compounds in combination with one or more pharmaceutically or

physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and/or preservatives. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, aerosol, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration.

A pharmaceutical composition may also, or alternatively, contain one or more active agents, such as drugs, which may be linked to a Glycomimetic compound or may be free within the composition. The attachment of an agent to a Glycomimetic compound may be covalent or noncovalent.

The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule or sponge that effects a slow release of modulating agent following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of modulating agent release. The amount of Glycomimetic compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Glycomimetic compounds are generally present within a pharmaceutical composition in a therapeutically effective amount. A therapeutically effective amount is an amount that results in a discernible patient benefit, such as a measured or observed response of a condition associated with *Pseudomonas* infection.

GLYCOMIMETIC COMPOUNDS METHODS OF USE

In general, Glycomimetic compounds described herein may be used for achieving diagnostic and/or therapeutic results in disease (e.g., human disease) involving infection by *Pseudomonas* (e.g., *P. aeruginosa*) bacteria. Such diagnostic and/or therapeutic results may be achieved *in vitro* and/or *in vivo* in an animal, preferably in a mammal such as a human, provided that *Pseudomonas* (e.g., *P.*

aeruginosa) is ultimately contacted with a Glycomimetic compound, in an amount and for a time sufficient to achieve a discernable diagnostic or therapeutic result. In the context of this invention, a therapeutic result would relate, for example, to the prevention of lung infections. In some conditions, therapeutic results would be associated with the inhibiting of *Pseudomonas* (including, for example, arresting the growth of or killing the bacteria or preventing colonization by the bacteria), such as *P. aeruginosa*. As used herein, therapy or therapeutic results includes treatment or prevention.

Glycomimetic compounds of the present invention may be administered in a manner appropriate to the disease to be treated or prevented. Appropriate dosages and a suitable duration and frequency of administration may be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the modulating agent(s) in an amount sufficient to provide treatment and/or prophylactic benefit. Within particularly preferred embodiments of the invention, a Glycomimetic compound may be administered at a dosage ranging from 0.001 to 1000 mg/kg body weight (more typically 0.01 to 1000 mg/kg), on a regimen of single or multiple daily doses. Appropriate dosages may generally be determined using experimental models and/or clinical trials. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

Glycomimetic compounds may also be used to target substances to *Pseudomonas* bacteria, e.g., *P. aeruginosa*. Such substances include therapeutic agents and diagnostic agents. Therapeutic agents may be a molecule, virus, viral component, cell, cell component or any other substance that can be demonstrated to modify the properties of a target cell so as to provide a benefit for treating or preventing a disorder or regulating the physiology of a patient. A therapeutic agent may also be a prodrug that generates an agent having a biological activity *in vivo*. Molecules that may be therapeutic agents may be, for example, polypeptides, amino acids, nucleic acids, polynucleotides, steroids, polysaccharides or inorganic compounds. Such molecules may function in any of a variety of ways, including as enzymes, enzyme inhibitors, hormones, receptors, antisense oligonucleotides, catalytic polynucleotides, anti-viral agents, anti-tumor agents, anti-bacterial agents, immunomodulating agents and cytotoxic agents (e.g., radionuclides such as iodine, bromine, lead, rhenium, homium, palladium or copper). Diagnostic agents include imaging agents such as metals and

radioactive agents (e.g., gallium, technetium, indium, strontium, iodine, barium, bromine and phosphorus-containing compounds), contrast agents, dyes (e.g., fluorescent dyes and chromophores) and enzymes that catalyze a colorimetric or fluorometric reaction. In general, therapeutic and diagnostic agents may be attached to a Glycomimetic compound using a variety of techniques such as those described above. For targeting purposes, a Glycomimetic compound may be administered to a patient as described herein.

Glycomimetic compounds may also be used *in vitro*, e.g., within a variety of well known cell culture and cell separation methods. For example, a Glycomimetic compound may be immobilized on a solid support (such as linked to the interior surface of a tissue culture plate or other cell culture support) for use in immobilizing *Pseudomonas* bacteria for screens, assays and growth in culture. Such linkage may be performed by any suitable technique, such as the methods described above, as well as other standard techniques. Glycomimetic compounds may also be used to facilitate cell identification and sorting *in vitro*, permitting the selection of such bacterial cells. Preferably, the Glycomimetic compound(s) for use in such methods is linked to a diagnostic agent which is a detectable marker. Suitable markers are well known in the art and include radionuclides, luminescent groups, fluorescent groups, enzymes, dyes, constant immunoglobulin domains and biotin. Within one preferred embodiment, a Glycomimetic compound linked to a fluorescent marker, such as fluorescein, is contacted with the cells, which are then analyzed by fluorescence activated cell sorting (FACS).

Such *in vitro* methods generally comprise contacting a sample (e.g., a biological preparation) with any one of the Glycomimetic compounds, and detecting the compound in the sample. If desired, one or more wash steps may be added to a method. For example, subsequent to contacting a sample with a Glycomimetic compound but prior to detection of the compound, the sample may be washed (i.e., contacted with a fluid and then removal of the fluid in order to remove unbound Glycomimetic compound). Alternatively, or in addition, a wash step may be added during the detection process. For example, if a Glycomimetic compound possesses a marker (a diagnostic agent) that can bind to a substance that is detectable, it may be desirable to wash the sample subsequent to contacting the sample with a detectable substance, but prior to the detection. As used herein, the phrase "detecting the compound (or agent) in the sample" includes detecting the compound (or agent) while it is bound to the sample, or detecting the compound (or agent) which was bound to the sample but after it has been separated from the sample.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1
SYNTHESIS OF 3 (FIGURE 3)

Formation of intermediate C:

Compounds A (5.00 g, 12.74 mmol) and B (4.50 g, 19.11 mmol) and NIS (3.58 g, 15.93 mmol) are dissolved in CH_2Cl_2 (50 ml) and cooled to 0°C. A solution of trifluoromethanesulfonic acid (0.15 M in CH_2Cl_2) is added dropwise with stirring. After the solution changes color from orange to dark brown, addition of TMS-OH ceases. The solution is then washed with saturated $NaHCO_3$ (30 ml) and the organic layer is dried with Na_2SO_4 and evaporated to dryness. The syrup obtained is purified by silica gel chromatography (hexane/ether, 1:1) and used in the next step.

The compound obtained previously is dissolved in THF (40 ml) and Pd (10%)/C (1/10 by mass) is added. The solution is degassed and an atmosphere of H_2 is generated. The reaction is allowed to proceed at RT until disappearance of starting material is confirmed by TLC. The solution is filtered thru a bed of celite and the filtrate is concentrated *in vacuo* giving the 4 and 6 OH compound. The compound is then dissolved in pyridine (25 ml) and cooled to 0°C. Ph_3CCl (1.2 eq) is added dropwise and the reaction is allowed to proceed at RT for 6 hrs. Ethyl acetate (50 ml) is then added and the solution is washed with 0.1N HCl (2 X 50 ml), saturated $NaHCO_3$ (1 X 50 ml) and saturated NaCl (1 X 50 ml). The organic layer is dried with Na_2SO_4 and evaporated to dryness. Intermediate C is obtained by silica gel chromatography.

Formation of 20:

Compound C (800 mg, 1.41 mmol) and Et_4NBr (353 mg, 1.69 mmol) are dissolved in DMF/CH_2Cl_2 (10 ml, 1:1, containing molecular sieves) and cooled to 0°C. Br_2 (298 mg, 1.86 mmol, in CH_2Cl_2) is added dropwise to a separate solution of compound D (808 mg, 1.69 mmol) in CH_2Cl_2 at 0°C. After 30 min the Br_2/D solution is quenched with cyclohexene (0.2 ml) and added to the C solution immediately (within 10 min). This mixture is allowed to react for 65 hrs at RT. Ethyl acetate (100 ml) is added, the solution filtered, and the filtrate is washed with saturated NaS_2O_3 (2 X 50 ml) and saturated NaCl (2 X 50 ml). The organic layer is dried with Na_2SO_4 and

evaporated to dryness. The resultant syrup is then dissolved in ether (50 ml) and formic acid (10 ml), is added with stirring. Upon completion of the reaction (as verified by TLC), the solution is washed with saturated $NaHCO_3$ (2 X 50 ml) and saturated NaCl (1 X 50 ml). The organic layer is then dried with Na_2SO_4, then evaporated to dryness. Compound 20 is then purified by silica gel chromatography.

Formation of intermediate F:

Compound 20 (1 g, 1.02 mmol) is dissolved in MeOH/dioxane (10 ml, 20:1) and NaOMe (0.10 mmol) is added with stirring. The reaction is allowed to proceed at 50°C for 20 hrs and then 2 drops of acetic acid are added. The solution is evaporated to dryness, dissolved in ethyl ether (25 ml) and washed with saturated NaCl (1 X 50 ml). The organic layer is dried with Na_2SO_4 and evaporated to dryness. The final product is purified by silica gel chromatography. The product (0.980 mmol) and Bu_2Sn (1.08 mmol) are suspended in MeOH (15 ml) and heated to reflux for 2 hrs. The resultant clear solution is then evaporated to dryness, taken up in pentane (10 ml) and evaporated giving a colorless foam. The foam is dissolved in 1,2-dimethoxyethane (DME, 15 ml), compound E (1.96 mmol) and CsF (1.18 mmol) are added and the reaction stirred for 2 hrs at room temperature. After 2 hrs, 1M KH_2PO_4 (50 ml) and KF (1 g) are added with stirring followed by extraction with ethyl acetate (2 X 25 ml). The organic layer is washed with 10% KF (2 X 50 ml) and saturated NaCl (2 X 50 ml), dried with Na_2SO_4 and evaporated to dryness under reduced pressure. Compound F is obtained via silica gel chromatography.

Formation of 3:

Compound F is dissolved in CH_3OH (50 ml) and Pd (10%)/C (1/10 by mass) is added. The solution is degassed and an atmosphere of H_2 is generated. The reaction is allowed to proceed at RT until disappearance of starting material is confirmed by TLC. The solution is filtered thru a bed of celite and the filtrate is concentrated *in vacuo* giving compound 3.

EXAMPLE 2
SYNTHESIS OF 21 (FIGURE 4)

Formation of intermediate H:

G (15.0 g, 66.9 mmol) and Bu_2SnO (20.0 g, 80.3 mmol) are suspended in MeOH (450 ml) and heated to reflux for 2 hrs. The resultant clear solution is then

evaporated to dryness, taken up in pentane and evaporated again giving a colorless foam. The foam is dissolved in 1,2-dimethoxyethane (DME, 120 ml), E (39.6 g, 100.3 mmol) and CsF (12.2 g, 80.3 mmol) are added and the reaction stirred for 2 hrs at room temperature. After 2 hrs, 1M KH_2PO_4 (700 ml) and KF (25 g) are added with stirring followed by extraction with ethyl acetate (3 X 250 ml). The organic layer is washed with 10% KF (2 X 250 ml) and sat. NaCl (1 X 250 ml), dried with Na_2SO_4 and evaporated to dryness under reduced pressure. The compound (19.3 g, 41.2 mmol) is purified by silica gel chromatography and immediately dissolved in pyridine (210 ml) with a crystal DMAP. The solution is cooled to 0°C and benzoyl chloride (52.1 g, 370.7 mmol) is added dropwise with stirring. The solution is allowed to warm to room temperature slowly and the reaction proceeds at RT for 20 min. The solution is evaporated to dryness, dissolved in ethyl acetate (500 ml), and washed with 0.1M HCl (2 X 250 ml), saturated $NaHCO_3$ (2 X 250 ml) and saturated NaCl (1 X 250 ml) solutions. The organic layer is dried with Na_2SO_4 and evaporated to dryness. H is obtained via silica gel chromatography.

Formation of intermediate I:

H (10.0 g, 12.82 mmol) and B (6.05 g, 25.64 mmol) are dissolved in CH_2Cl_2 (75 ml) and 0.15M CF_3SO_3H (in CH_2Cl_2) is added dropwise at -10°C with stirring. Addition is stopped when the orange solution changes to brown. Ethyl acetate (500 ml) is added and the solution is washed with sat $NaHCO_3$ (4 X 250 ml) and saturated NaCl (250 ml). The organic layer is then dried with Na_2SO_4 and evaporated under reduced pressure. The compound (7.96 g, 9.19 mmol) is then purified by silica gel chromatography and then dissolved in DMF (55 ml). TBDMS-Cl (1.52 g, 10.1 mmol) and imidazole (0.94 g, 13.8 mmol) are then added and the reaction allowed to proceed at RT for 1 hr. Ethyl acetate (250 ml) is added and the solution washed with saturated $NaHCO_3$ (5 X 250 ml) and saturated NaCl (1 X 250 ml). The organic layer is then dried with Na_2SO_4 and purified by silica gel chromatography giving compound I.

Formation of intermediate J:

Compound I (7.71 g, 7.87 mmol) and Et_4NBr (2.00 g, 9.45 mmol) are dissolved in DMF/CH_2Cl_2 (60 ml, 1:1, containing molecular sieves-12 g) and cooled to 0°C. Br_2 (1.90 g, 11.8 mmol) in CH_2Cl_2 (11 ml) is added dropwise to a separate solution of compound D (4.5 g, 9.45 mmol) in CH_2Cl_2 at 0°C. After 30 min, the Br_2/D solution is quenched with cyclohexene (2.5 ml) and added to the I solution immediately (within 10 min). This mixture is allowed to react for 65 hrs at RT. CH_2Cl_2 (250 ml) is

added, the solution filtered, and the filtrate is washed with saturated $NaHCO_3$ (2 X 50 ml), 0.5M HCl (2 X 250 ml) and saturated NaCl (2 X 250 ml). The organic layer is dried with Na_2SO_4 and evaporated to dryness. The mixture is dissolved in MeCN (85 ml) at RT and a solution of Et_3N (0.21 ml) and H_2SiF_6 (1.3 ml, 35 %) in MeCN (17 ml) is added and stirred for 2 hrs. CH_2Cl_2 (250 ml) is added and the solution washed with saturated $NaHCO_3$ (3 X 250 ml) and saturated NaCl (1 X 250 ml). The organic layer is dried with Na_2SO_4, evaporated to dryness and J is purified by silica gel chromatography.

Formation of intermediate K:

J (12.5 g, 9.75 mmol) is dissolved in pyridine (80 ml) and methanesulfonylchloride (3.35 g, 29.2 mmol) is added dropwise with stirring over 5 min. The reaction is allowed to proceed for 30 min and then ethyl acetate (500 ml) is added. The solution is washed with 1N HCl (250 ml). The organic layer is dried with Na_2SO_4 and evaporated. The resultant syrup (12.95 g, 9.52 mmol) is dissolved in DMF (40 ml) and NaN_3 (4.64 g, 74.4 mmol) is added. The reaction is allowed to proceed for 35 hrs under argon atmosphere at 65°C. The solution is diluted with ethyl acetate (500 ml) and washed with H_2O (300 ml) and saturated NaCl (150 ml). The organic layer is dried with Na_2SO_3 and evaporated to dryness. The compound is purified by silica gel chromatography. The purified product (12.2 g, 9.33 mmol) is then suspended in MeOH/H_2O (200 ml/20 ml) solution and LiOH-H_2O (5.1 g, 121.3 mmol) was added. The reaction is allowed to proceed at 65°C for 20 hrs. Ethyl ether (500 ml) was added and the solution is washed with saturated NaCl (200 ml). The organic layer is dried with Na_2SO_4 and evaporated to dryness. Compound K is purified via silica gel chromatography.

Formation of 21:

Compound K (8.45 g, 9.33 mmol) is dissolved in dioxane/H_2O (250 ml/50 ml) and Pd (10%)/C (3.4 g) is added. The solution is degassed and an atmosphere of H_2 is generated. The reaction is allowed to proceed at RT until disappearance of starting material is confirmed by TLC. The solution is filtered thru a bed of celite and the filtrate is concentrated *in vacuo* giving compound 21.

EXAMPLE 3

SYNTHESIS OF 15 (FIGURE 5)

Formation of intermediate L:

Compound 20 (10 mmol) is dissolved in CH_2Cl_2 (30 ml) and DMSO (20 mmol) is added and the solution is cooled to -60°C. Oxalyl chloride (11 mmol) is added slowly to the stirred solution of 20. The reaction is allowed to proceed for 30 min under N_2 atmosphere. The reaction is washed with 0.1M HCl, saturated $NaHCO_3$, and saturated NaCl. The organic layer is dried with Na_2SO_4 and evaporated to dryness. The resultant syrup is placed in tBuOH (20 ml) and 2-methyl-2-butene (10 ml) and NaH_2PO_4 (20 mmol) is added with stirring. The reaction is allowed to proceed for 3 hrs and is then evaporated taken up in CH_2Cl_2 and washed with 0.1M HCl, saturated $NaHCO_3$, and saturated NaCl. The resultant compound is purified by silica gel chromatography giving compound L.

Formation of intermediate N:

Compound L (10 mmol) is dissolved in DMF (15 ml) and compound M (10 mmol) , HBTU (12 mmol) and Et_3N (20 mmol) are added with stirring. The reaction is allowed to proceed at RT for 24 hrs. Ethyl acetate (100 ml) is added and the solution is washed with 0.1M HCl (1 X 100 ml), saturated $NaHCO_3$ (1 X 100 ml), and saturated NaCl (1 X 100 ml). The organic layer is dried with Na_2SO_4 and evaporated to dryness. Compound N is isolated via silica gel chromatography.

Formation of intermediate O:

Compound N (10 mmol) is dissolved in MeOH (35 ml) and NaOMe (1 mmol) is added with stirring. The reaction is allowed to proceed at RT for 20 hrs. The solution is evaporated to dryness, dissolved in ethyl ether (50 ml) and washed with saturated NaCl (1 X 50 ml). The organic layer is dried with Na_2SO_4 and evaporated to dryness. The final product is purified by silica gel chromatography. The product (0.980 mmol) and Bu_2Sn (1.08 mmol) are suspended in MeOH (15 ml) and heated to reflux for 2 hrs. The resultant clear solution is then evaporated to dryness, taken up in pentane (10 ml) and evaporated giving a colorless foam. The foam is dissolved in 1,2-dimethoxyethane (DME, 15 ml), compound E (1.96 mmol) and CsF (1.18 mmol) are added and the reaction stirred for 2 hrs at room temperature. After 2 hrs, 1M KH_2PO_4 (50 ml) and KF (1 g) are added with stirring followed by extraction with ethyl acetate (2 X 25 ml). The organic layer is washed with 10% KF (2 X 50 ml) and saturated NaCl (2

X 50 ml), dried with Na_2SO_4 and evaporated to dryness under reduced pressure. Compound O is obtained via silica gel chromatography.

Formation of 15:

Compound O (9 mmol) is dissolved in MeOH (200 ml) and Pd (10%)/C (3 g) is added. The solution is degassed and an atmosphere of H_2 is generated. The reaction is allowed to proceed at RT until disappearance of starting material is confirmed by TLC. The solution is filtered thru a bed of celite and the filtrate is concentrated *in vacuo* giving compound 15.

EXAMPLE 4

ACYLATION OF 21 (FIGURE 6)

Reaction of 21 with acid chlorides:

Compound 21 (20 mg, 0.033 mmol) is dissolved in a THF/H_2O (2 ml, 1:1) solution containing 1N NaOH (pH adjusted between 8-10) and is cooled to 0°C. Cyclohexyl-carbonylchloride (0.049 mmol) is then added dropwise with stirring. The reaction is allowed to continue at 0°C for 3 hrs. The solution is quenched with ice and the solution is evaporated to dryness. Compound 1 is purified by reverse phase chromatography.

Reaction of 21 with isocyanates:

Compound 21 (30 mg, 0.049 mmol) is dissolved in a 0.5N aqueous NaOH solution (1 ml) and cooled to 0°C. Ethyl isocyanate (1.2 eq) is then added dropwise with stirring. The reaction is allowed to continue at RT for 3 hrs. The solution is quenched with ice and the solution is evaporated to dryness. Compound 2 is purified by reverse phase chromatography.

Reaction of 21 with chloro-orthoformates:

Compound 21 (20 mg, 0.033 mmol) is dissolved in a THF/H_2O (2 ml, 1:1) solution containing NaOH (pH adjusted between 8-10) and is cooled to 0°C. Benzyl-chloro-orthoformate (0.049 mmol) is then added dropwise with stirring. The reaction is allowed to continue at 0°C for 3 hrs. The solution is quenched with ice and the solution is evaporated to dryness. Compound 11 is purified by reverse phase chromatography.

Reaction of 21 with sulfonyl chlorides:

Compound 21 (20 mg, 0.033 mmol) is dissolved in a saturated aqueous $NaHCO_3$/toluene (2 ml, 1:1) solution and is cooled to 0°C. *p*-Toluenesulfonyl chloride (0.049 mmol) is then added dropwise with stirring. The reaction is allowed to continue at 0°C for 3 hrs. The solution is quenched with ice and the solution is evaporated to dryness. Compound 9 is purified by reverse phase chromatography.

EXAMPLE 5
SYNTHESIS OF COMPOUND 22 (FIGURE 7)

Compound 1 (2.77 mmol) is dissolved in a solution of diethyl ether/dichloromethane (1 ml, 1:1 mix) containing compound 2 (5.55 mmol). A 0.1M TMSOTf (2.8 ml) solution is then added dropwise with stirring. After 30 min the solution is neutralized by addition of sodium bicarbonate (0.5 g), filtered and concentrated to dryness. Compound 3 is purified via column chromatography (toluene/acetone, 4:1 mix).

Compound 3 (1.96 mmol) is stirred in DMF/dichloromethane (50 ml, 1:5 mix) along with 4A molecular sieves, TBABr (968 mmol) and $CuBr_2$ (529 mmol) for 1 hr. Compound 4 (2.94 mmol) is dissolved in dichloromethane (5 ml) and added dropwise to the solution. The solution is allowed to react for 24 hrs, filtered, and washed with saturated sodium bicarbonate and water (50 ml each). The organic layer is isolated, dried over sodium sulfate, and evaporated to dryness. Compound 5 is purified via column chromatography (hexane/ethyl acetate, 4:1 mix).

Compound 22 is obtained by dissolution of compound 5 in methanol followed by addition of 1M sodium methoxide solution. The solution is allowed to react for 3-5 hrs and is then neutralized with Amberlite IR-120 resin, filtered and concentrated in vacuo. Compound 22 is purified by column chromatography (dichloromethane/methanol. 20:1 mix).

EXAMPLE 6
SYNTHESIS OF COMPOUNDS 4, 6, 7, 8, 10, 12, 13, 14, 16, 17, 18, 19 AND 20

Starting from compound 21 (Example 2; Figure 4) these compounds are synthesized by following the procedures described in the literature (*Helvetica Chimica Acta* Vol. 83, pp. 2893-2907, 2000; *Angew Chem. Int Ed.* Vol. 40, No. 19, pp. 3644-3647, 2001.)

EXAMPLE 7

SYNTHESIS OF COMPOUNDS 23, 24, 25, 26, 27 AND 28

Synthesis of intermediates of 23: Compound i (2.77 mmol) is dissolved in a solution of diethylether/dichloromethane (1 ml, 1:1 mix) containing compound 2 (5.55 mmol). A 0.1 M TMSOTf (2.8 ml) solution is then added dropwise with stirring. After 30 min the solution is neutralized by addition of sodium bicarbonate (0.5 g), filtered and concentrated to dryness. 3 is purified by silica gel chromatography to give compound iii.

Compound iii (1.96 mmol) is stirred in DMF/dichloromethane (50 ml, 1:5 mix) along with 4A molecular sieves, TBABr (968 mmol) and $CuBr_2$ (529 mmol) for 1 hr. Compound xv (2.94 mmol) is dissolved in dichloromethane (5 ml) and added dropwise to the solution. The solution is allowed to react for 24 hr, filtered, and washed with saturated sodium bicarbonate and water (50 ml each). The organic layer is isolated, dried over sodium sulfate, and evaporated to dryness. iv is then purified by silica gel chromatography.

Compound iv is de-O-acetylated with 0.1N NaOMe in MeOH to give v and then treated with dibutyltin oxide in MeOH under refluxing condition to afford vi (after evaporation of the solvent) which is used for the next step without further purification.

Compound vi (1 mmol) is dissolved in DME (20 ml) and then added compound vii (2.5 mmol) and CsF (1.4 mmol). The resulting reaction mixture is stirred at room temperature for 8 hr, diluted with ethyl acetate and the organic layer is washed with water. The organic layer is concentrated to dryness and purified by silica gel chromatography to afford compound viii.

Synthesis of compound 23: Compound viii is hydrogenated in the presence of palladium in carbon to afford compound 23.

Synthesis of intermediate xx: Starting from N-acetyl glucosamine (5, 50 g) compound xx (50% overall yield) is synthesized following published procedures (*Bioorg. Med. Chem. Lett.* 11, pp. 923-925, 2001; *Carbohydr. Res.* 197, 75, 1990).

Synthesis of intermediate xv: Compound xv (15 g) is prepared from L-fucose following the procedure described in the literature (*Carbohydr. Res.* 201, 15-30, 1990).

Synthesis of intermediate xix: Compound xix is prepared from commercially available β-D-galactose-pentaacetate as described (WO 9701569; *Chem. Astr.*, 126 186312, 1997).

Synthesis of intermediate xxxiv: Commercially available N-acetyl neuraminic acid (xxx, 10 g) is suspended in MeOH-H_2O (60 ml, 9:1) and the pH is adjusted to 8.1 by adding an aqueous solution of cesium carbonate. The solvent is removed and the residue is repeatedly evaporated with ethanol and then with hexane. The material is dissolved in DMF (65 ml) and benzyl bromide (3.5 ml) is added within 20 min. After the mixture is stirred for 16 hr, dichloromethane (100 ml) is added and washed with water (50 ml). The solvent is evaporated off and purified by silica gel chromatography to give xxxi in 68% yield.

To a solution of compound xxxi (7 g) in pyridine (50 ml) is added acetic anhydride (48 ml) and the reaction mixture is stirred at RT for 16 hr. Solvent is evaporated off and the residue (xxxii) is dissolved in dry DMF (25 ml). To the mixture is added powdered ammonium carbonate (2 g) and the mixture is stirred for 12 hr at 28 degree centigrade. The mixture is added to a ice-cold solution of 1N HCl in water (50 ml) and dichloromethane (100 ml) is added. After solvent extraction, organic layer is evaporated off and then dried under vacuum for 24 hr. The residue is purified by silica gel chromatography to afford xxxiii in 71% yield.

Compound xxxiii is dissolved in dry dichloromethane and 2,6-di-tert-butyl-pyridine (5 g) is added. The solution is cooled down to -20 degree centigrade and trifluoromethanesulfonic anhydride (7 g) is added portionwise in 10 min. The mixture is stirred for 4 hr and diluted with dichloromethane (100 ml) and is added to a solution of potassium hydrogen phosphate (500 ml). The layers are separated and the organic layer is dried (sodium sulfate) and solvent is evaporated off to afford xxxiv which is used for the next step without further purification.

Synthesis of intermediate xxxv: To a mixture of compound xx (10 g) and compound xv (15 g) in dichloromethane (100 ml) is molecular sieves (4A, 8 g). After stirring at RT for 1 hr, tetraethylammonium bromide (5 g) is added. A solution of bromine (1 g) in dichloromethane (25 ml) is added dropwise during 1 hr. The reaction mixture is continued to stir for 3 hr, filtered through a bed of celite and washed successively with cold water, saturated solution of sodium bicarbonate and water. Solvent is evaporated off and subjected to silica gel chromatography.

The product was treated with sodium cyanoborohydride in THF and HCl in ether to afford compound xxxv in 70% overall yield after silica gel chromatography.

Synthesis of intermediate xxxvi: To a mixture of xxxv (10 g) and xix (7 g) in dichloromethane (80 ml) is added N-iodosuccinimide (15 g) and molecular sieves (4A, 8 g). The reaction mixture is placed in an ice bath. The solution is stirred at 0-5 degree for 30 min and a solution of triflic acid (0.2 ml) in dichloromethane (25 ml)

is added dropwise during 1 hr with stirring. Stirring is continued for 2 hr, filtered through a bed of celite and washed successively with cold water, cold saturated solution of sodium bicarbonate and cold water. Solvent is evaporated off and purified by silica gel chromatography to afforded xxxvi in 68% yield.

Synthesis of intermediate xxxvii: Compound xxxvi (8 g) is treated with 0.05N NaOEt in MeOH (100 ml) for 4 hr and after neutralization with IR120 (hydrogen form) resin, the reaction mixture is filtered off. The solvent is evaporated off to afford compound xxxvii in 96% yield.

Synthesis of intermediate xxxviii: Compound xxxvii (5 g) is treated with dibutyltinoxide (1 g) in MeOH for 4 hr under reflux. The solvent is evaporated off and co-evaporated with toluene several times and finally the residue is dried under high vacuum for 24 hr.

The crude reaction mixture is dissolved in dimethoxyetahne (DME, 100 ml) and is added CsF (1.7 g), and compound xxxiv (2.5 g). The reaction mixture is stirred at RT for 8 hr and ethyl acetate (100 ml) is added. Organic layer is washed water and organic solvent is evaporated off. The product is purified by silica gel chromatography to afford xxxviii in 64% yield.

Synthesis of intermediate xxxix: Compound xxxviii (2 g) is de-O-acetylated with 0.01N NaOMe in MeOH (100 ml, 1 hr), the crude reaction mixture is neutralized with IR120 (hydrogen form) resin, and solvent is evaporated off.

Product from the above reaction is dissolved in dioxane-water (1:1, 50 ml) and 10% PD-C is added. The reaction mixture is stirred vigorously under hydrogen atmosphere for 22 hr, filtered through a bed of celite and the solvent is evaporated off. Silica gel chromatography of resulting syrup to afford xxxix in 77% yield.

Synthesis of compound 24: To a solution of compound xxxix in MeOH-H_2O is added a solution of NaOMe in MeOH and the reaction mixture is stirred for 2 hr at room temperature. Neutralization with IR 120 resin and evaporation to dryness affords compound 24.

Synthesis of xxxx: Compound xxxix is treated (500 mg) with ethylenediamine at 70 degree centigrade for 4 hr. Solvent is evaporated off and the syrupy residue is purified by silica gel chromatography to give compound xxxx in 77 % yield.

Synthesis of compound xxxxv: 3-nitro-benzyl iodide is added to an aqueous solution (pH 11) of commercially available 8-aminonaphthalene-1,3,5-trisulfonic acid (xxxxxi) with stirring at room temperature. pH of the solution is

adjusted to 1 and after evaporation of the solvent, the product xxxxiii is precipitated out from ethanol .

Platinum catalyzed hydrogenation of compound xxxxiii affords compound xxxxiv in 96% yield.

To a solution of compound xxxxiv in phosphate buffer (pH 7.1) is added commercially available squaric acid and the reaction mixture is stirred for 4 hr at RT. It is then purified by reverse phase HPLC to afford compound xxxxv.

Synthesis of compound 25: Compound xxxx (0.2 g) is dissolved in carbonate buffer (2 ml, pH 8.8) and compound xxxxv (0.4 g) is added. The reaction mixture is stirred at RT for 24 hr. Another batch (0.2 g) of compound xxxxv is added and stirring is continued for 20 h at RT. Solvent is evaporated off and the mixture is purified by reverse phase HPLC to afforded 25.

Synthesis of compound 26: Compound xxxix (0.1 g) is reacted with compound xxxxiv to afford compound 26 after purification by HPLC.

Synthesis of intermediate xxxxxii: Commercially available compound xxxxvi (4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzoic acid), 1 eq) and KOAc (3 equ) are placed in THF (25 ml). To the resulting slurry is added $PdCl_2$ and commercially available p-bromonitrobenzene (xxxxvii, 1.2 equ) with stirring and the mixture is gently heated to 80 degree centigrade. After 6 hr, the reaction mixture is evaporated to dryness, dissolved in dichloromethane (30 ml) and washed with distilled water and saturated solution of sodium bicarbonate. The resulting biphenyl compound xxxxviii is taken directly to the next step.

Compound xxxxviii, (1 eq), dimethylaminopyridine (catalytic amount, one crystal), and EDCl (1.05 eq) are dissolved in DMF or THF (20 ml) and allowed to react at RT for 10 min. Commercially available compound xxxxix (8-aminonaphthalene-1,3,5-trisulfonic acid) is added to the reaction mixture with stirring and the reaction is allowed to proceed at RT under nitrogen for 48 hr. The reaction mixture is then evaporated to dryness and purified by reverse phase chromatography to afforded compound xxxxx.

To a solution of compound xxxxx in EtOAc is added PD-C and the reaction mixture is stirred under an atmosphere hydrogen for 2 hr. The reaction mixture is filtered through a bed of celite and evaporated to dryness to afford compound xxxxxi.

To a solution of compound xxxxxi in phosphate buffer (pH 7.1) is added commercially available squaric acid and the reaction mixture is stirred for 4 hr at RT. It is then purified by reverse phase HPLC to afford compound xxxxxii.

Synthesis of compound 27: Compound xxxx (0.2 g) is dissolved in carbonate buffer (2 ml, pH 8.8) and compound xxxxxii (0.4 g) is added. The reaction mixture is stirred at RT for 24 hr. Another batch (0.2 g) of compound xxxxxii is added and stirring is continued for 20 h at RT. Solvent is evaporated off and the mixture is purified by reverse phase HPLC to afford 27.

Synthesis of compound 28: Compound xxxix is reacted with compound xxxxxi to afford compound 28 which is purified by HPLC.

SYNTHETIC REFERENCES:

The synthesis protocols for preparation of certain of the compounds included within this application are illustrated in the following references: *Helvetica Chemica Acta* Vol. 83, pp.2893-2907 (2000) and *Angew. Chem. Int. Ed.* Vol. 40, No. 19, pp. 3644-3647 (2001).

EXAMPLE 8
ASSAYS

Purified oligosaccharides chemically coupled to albumin (neoglycoproteins) are coated in plastic microtiter wells. After blocking with BSA, the wells are then incubated and allowed to bind to purified PA-IIL lectin. Bound PA-IIL lectin is detected with anti-PA-IIL rabbit antisera followed by HRP-labeled anti-rabbit Ig and TMB reagent with color development by 1M H_3PO_4 (Figure 18). Structures and activities are presented in Table 1.

Table 1. Structures of immobilized neutral oligosaccharides screened for binding to PA-IIL lectin.

Results of screening immobilized neutral oligosaccharides are described in Figure 18 and summarized below. Type 1 lacto-series chains (e.g., Le^a structures) binding better to PA-IIL lectin than lacto-series type 2 chains (e.g., Le^x structures). Extension of the type 1 structure as in Le^a/Le^x results in the most active compound in this series.

Carbohydrate Structure	Name	PA-IIL binding
Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R	LNFI (H-type 1)	+
Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 4 Fucα1	Lea	++
Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 3 Fucα1	Ley	+
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 3 3 Fucα1 Fucα1	DiLex	+
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 3 3 3 Fucα1 Fucα1 Fucα1	TriLex	+
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R	LNnT	-
Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-R 4 3 Fucα1 Fucα1	Lea/Lex	+++
Galβ1-3GalNAcβ1-4Galβ1-4Glc-R	Gangliotetraose	-

Table 2. Structures of immobilized acidic oligosaccharides screened for binding to PA-IIL lectin

Results of screening immobilized acidic oligosaccharides are described in Figure 19 and summarized below. Only those sialylated oligosaccharides containing fucose will bind PA-IIL lectin. The second sialyl group on disialyl Le^a appears to inhibit binding.

Carbohydrate Structure	Name	PA-IIL binding
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 4 Fucα1	SialylLea	++
Neu5Acα2 6 Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 4 Fucα1	DiSialylLea	+
Neu5Acα2 6 Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R	DiSialylLNT	-
Neu5Acα2-3Galβ1-3GlcNAcb1-3Galβ1-4Glc-R	LSTa	-
Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R 6 Neu5Acα2	LSTb	-
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-R	LSTa	-
Neu5Acα2-3Galβ1-4Glc-R	3'SL	-
Neu5Acα2-6Galβ1-4Glc-R	6'SL	-

Purified oligosaccharides chemically coupled to albumin (neoglycoproteins) are coated in plastic microtiter wells. After blocking with BSA, the wells are then incubated and allowed to bind to purified PA-IIL lectin. Bound PA-IIL lectin is detected with anti-PA-IIL rabbit antisera followed by HRP-labeled anti-rabbit Ig and TMB reagent with color development by 1M H_3PO_4 (Figure 19). Structures and activities are presented in Table 2.

Biotinylated polymers of Fucose (Fuc-PAA-biotin, GlycoTech Corp.) are allowed to couple HRP-labeled streptavidin (KPL labs) overnight at 4°C. PA-IIL

lectin is immobilized in plastic microtiter wells and allowed to react with the HRP-labeled fucosylated polymer. Binding is detected by TMB substrate followed by color development with 1M H_3PO_4 (Figure 20). Conditions chosen for the assay are coating with 3 μg/ml PA-IIL and incubation of inhibitor with 2 μg/ml of fucosylated polymer.

Compound 23 inhibited PA-IIL with an IC_{50} of 420 nM (Figure 22), while native saccharide inhibitor, Mannose, inhibited PA-IIL lectin with an IC_{50} of 95 μM and the negative control saccharide, Galactose, showed no inhibition (data not shown).

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

CLAIMS

1. A method of inhibiting *Pseudomonas* bacteria in a warm-blooded animal comprising administering to the animal in an amount effective to inhibit the bacteria a compound comprising a compound according to Figure 1 or Figure 2.

2. The method of claim 1 wherein the compound is linked to a therapeutic agent.

3. The method of claim 1 or claim 2 wherein the compound is in combination with a pharmaceutically acceptable carrier or diluent.

4. The method of claim 1 wherein the bacteria are *Pseudomonas aeruginosa*.

5. A conjugate comprising a therapeutic agent linked to a compound according to Figure 1 or Figure 2.

6. The conjugate of claim 5 wherein the conjugate is in combination with a pharmaceutically acceptable carrier or diluent.

7. A method of detecting *Pseudomonas* bacteria comprising contacting a sample with a diagnostic agent linked to a compound comprising a compound according to Figure 1 or Figure 2, under conditions sufficient for the compound to bind to the bacteria if present in the sample; and detecting the agent present in the sample, wherein the presence of agent in the sample is indicative of the presence of *Pseudomonas* bacteria.

8. The method of claim 7 wherein the bacteria are *Pseudomonas aeruginosa*.

9. A method of immobilizing *Pseudomonas* bacteria on a solid support comprising contacting, under conditions sufficient for binding, a sample containing *Pseudomonas* bacteria with a compound comprising a compound according to Figure 1 or Figure 2 that is immobilized on a solid support; and separating the sample from the solid support.

10. A compound comprising a compound according to Figure 1 or Figure 2 for use in a method of inhibiting *Pseudomonas* bacteria.

11. The compound of claim 10 in combination with a pharmaceutically acceptable carrier or diluent.

12. The compound of claim 10 or claim 11 wherein the bacteria are *Pseudomonas aeruginosa*.

13. A conjugate according to claim 5 or claim 6 for use in a method of inhibiting *Pseudomonas* bacteria.

14. The conjugate of claim 13 wherein the bacteria are *Pseudomonas aeruginosa*.

15. Use of a compound comprising a compound according to Figure 1 or Figure 2 in the preparation of a medicament for the inhibition of *Pseudomonas* bacteria.

16. Use of a conjugate according to claim 5 in the preparation of a medicament for the inhibition of *Pseudomonas* bacteria.

17. The use according to claim 15 or claim 16 wherein the bacteria are *Pseudomonas aeruginosa*.

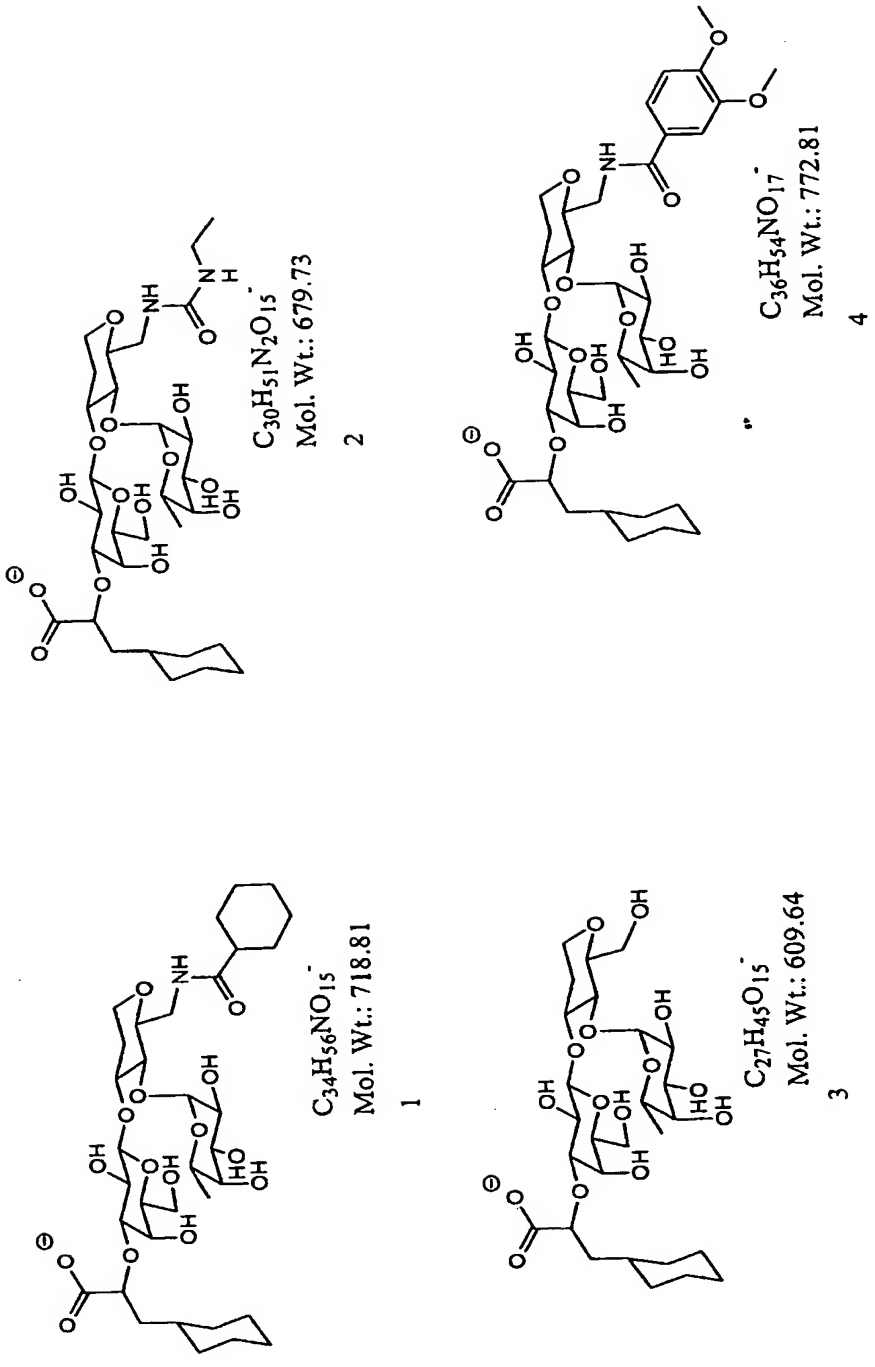

$C_{34}H_{56}NO_{15}^-$
Mol. Wt.: 718.81
1
$C_{30}H_{51}N_2O_{15}^-$
Mol. Wt.: 679.73
2
$C_{27}H_{45}O_{15}^-$
Mol. Wt.: 609.64
3
$C_{36}H_{54}NO_{17}^-$
Mol. Wt.: 772.81
4

FIG. 1A

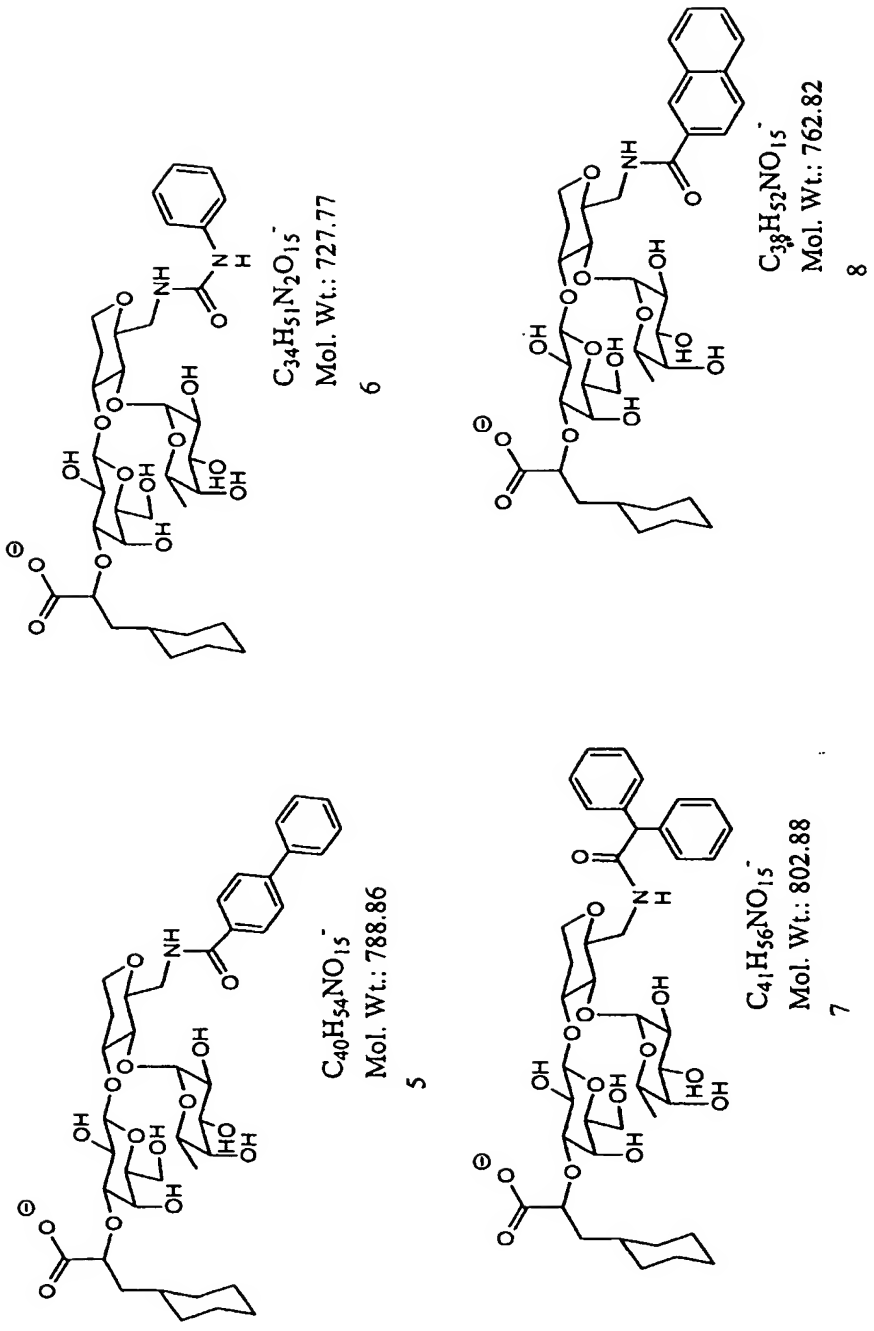

$C_{34}H_{51}N_2O_{15}^-$
Mol. Wt.: 727.77
6
$C_{38}H_{52}NO_{15}^-$
Mol. Wt.: 762.82
8
$C_{40}H_{54}NO_{15}^-$
Mol. Wt.: 788.86
5
$C_{41}H_{56}NO_{15}^-$
Mol. Wt.: 802.88
7

FIG. 1B

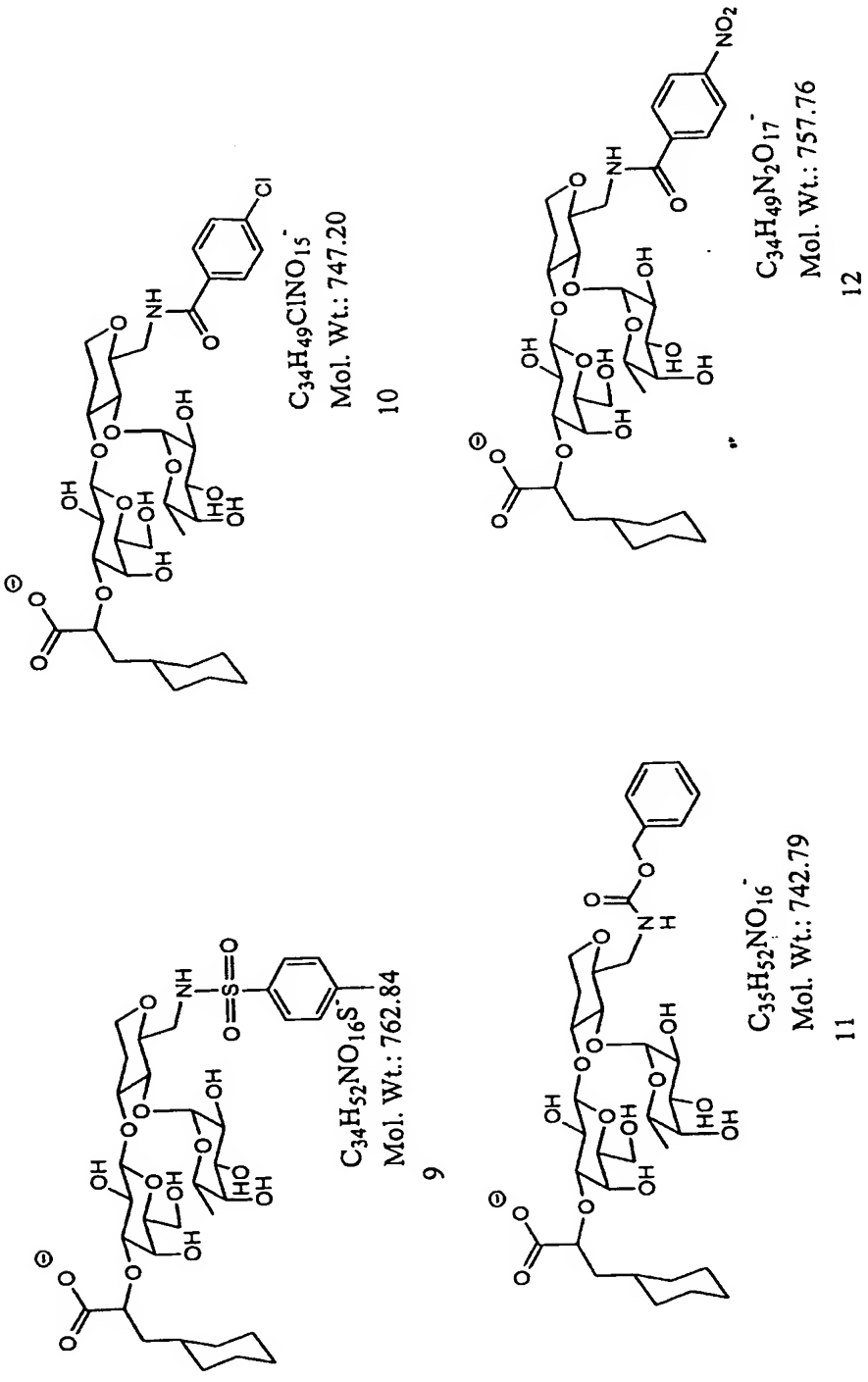
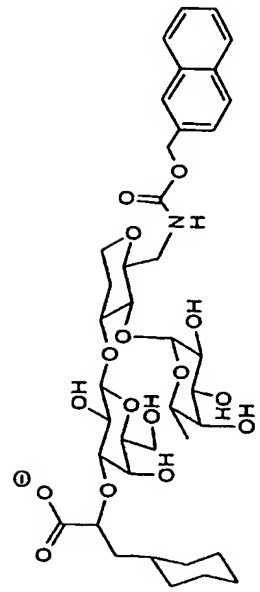
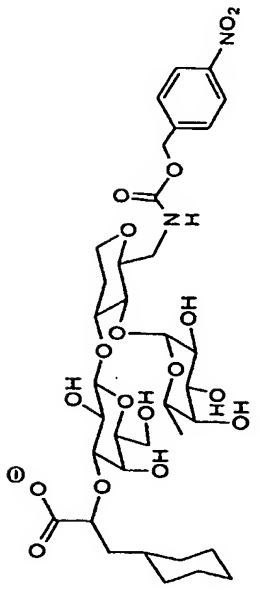

$C_{34}H_{49}ClNO_{15}^-$
Mol. Wt.: 747.20
10
$C_{34}H_{49}N_2O_{17}^-$
Mol. Wt.: 757.76
12
$C_{34}H_{52}NO_{16}S^-$
Mol. Wt.: 762.84
9
$C_{35}H_{52}NO_{16}^-$
Mol. Wt.: 742.79
11

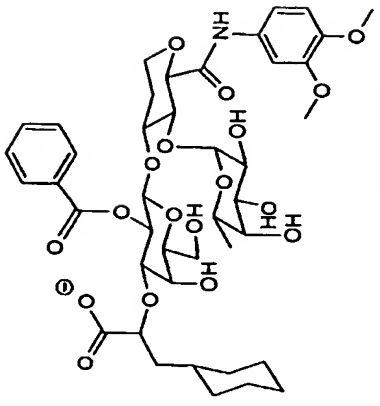
FIG. 1C



$C_{39}H_{54}NO_{16}^-$
Mol. Wt.: 792.84
14



$C_{35}H_{51}N_2O_{18}^-$
Mol. Wt.: 787.78
13



$C_{42}H_{56}NO_{18}^-$
Mol. Wt.: 862.89
15

FIG. 1D

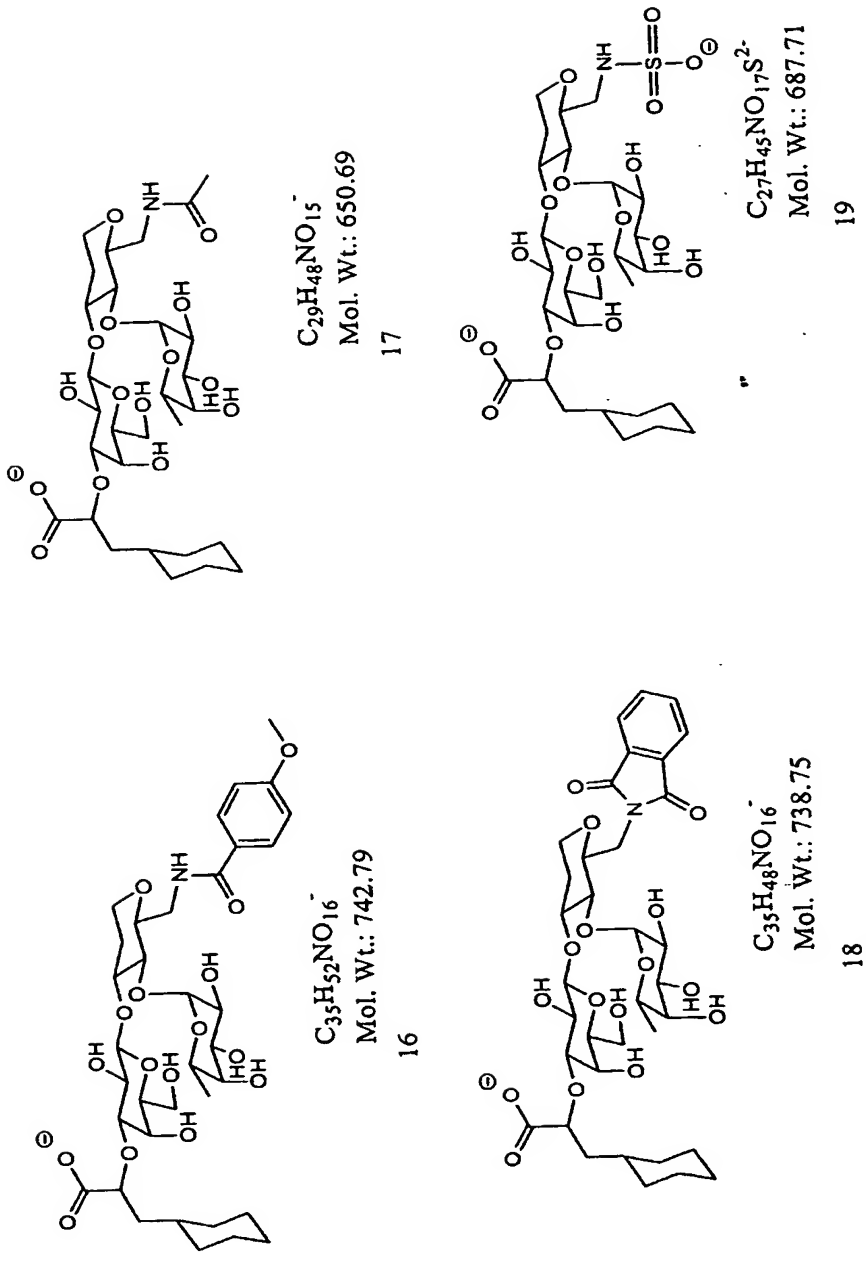
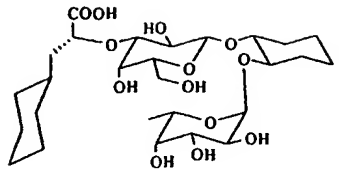
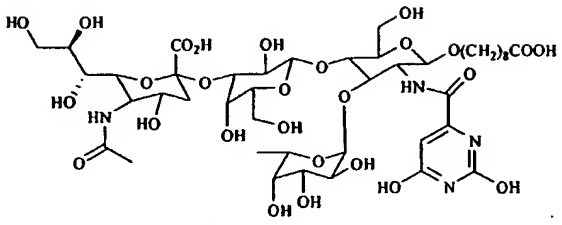

$C_{29}H_{48}NO_{15}^-$
Mol. Wt.: 650.69
17
$C_{27}H_{45}NO_{17}S^{2-}$
Mol. Wt.: 687.71
19
$C_{35}H_{52}NO_{16}^-$
Mol. Wt.: 742.79
16
$C_{35}H_{48}NO_{16}^-$
Mol. Wt.: 738.75
18

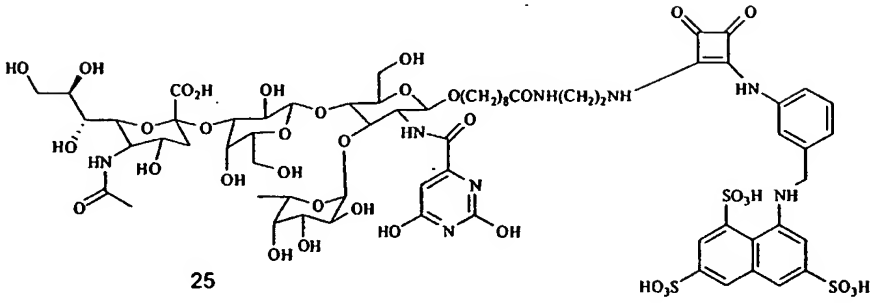
FIG. 1E



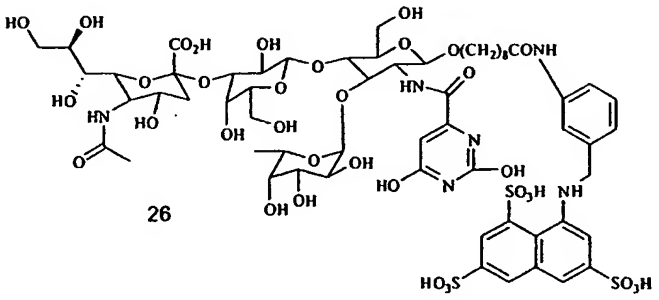
23



24

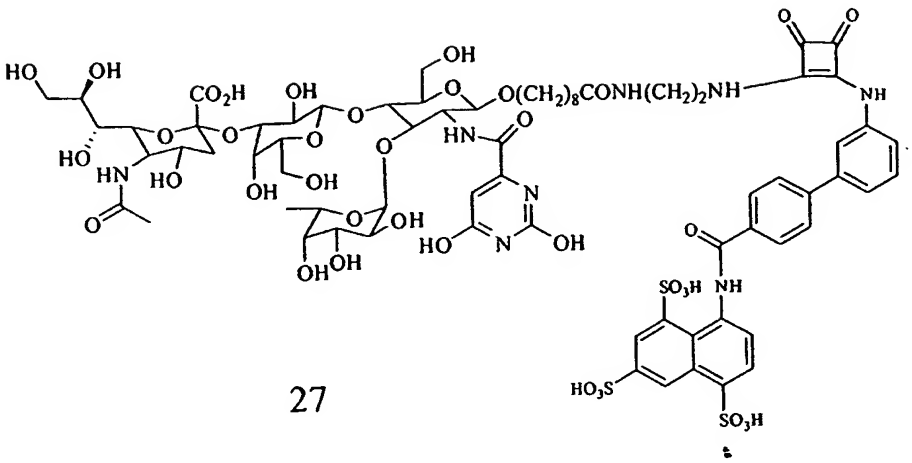


25

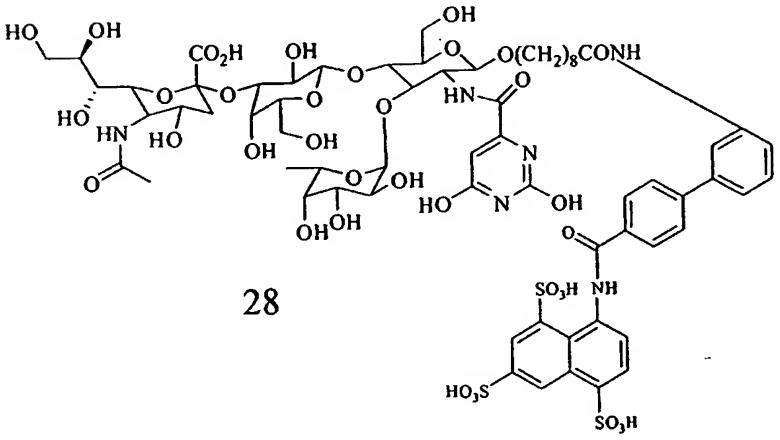


26

FIG. 2A



27



28

FIG. 2B

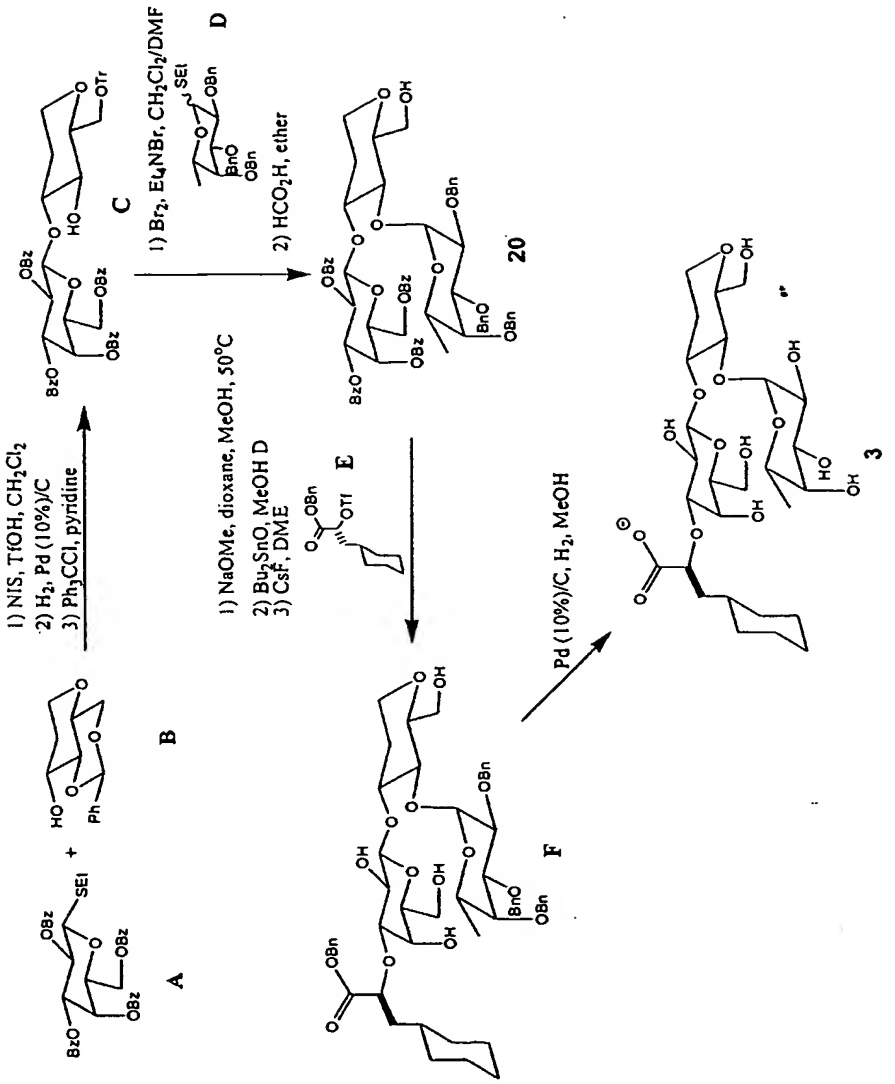

1) NIS, TfOH, CH_2Cl_2
2) H_2, Pd (10%)/C
3) Ph_3CCl, pyridine
1) Br_2, Et_4NBr, CH_2Cl_2/DMF
2) HCO_2H, ether
1) NaOMe, dioxane, MeOH, 50°C
2) Bu_2SnO, MeOH D
3) CsF, DME
Pd (10%)/C, H_2, MeOH
A
B
C
D
E
F
20
3

FIG. 3

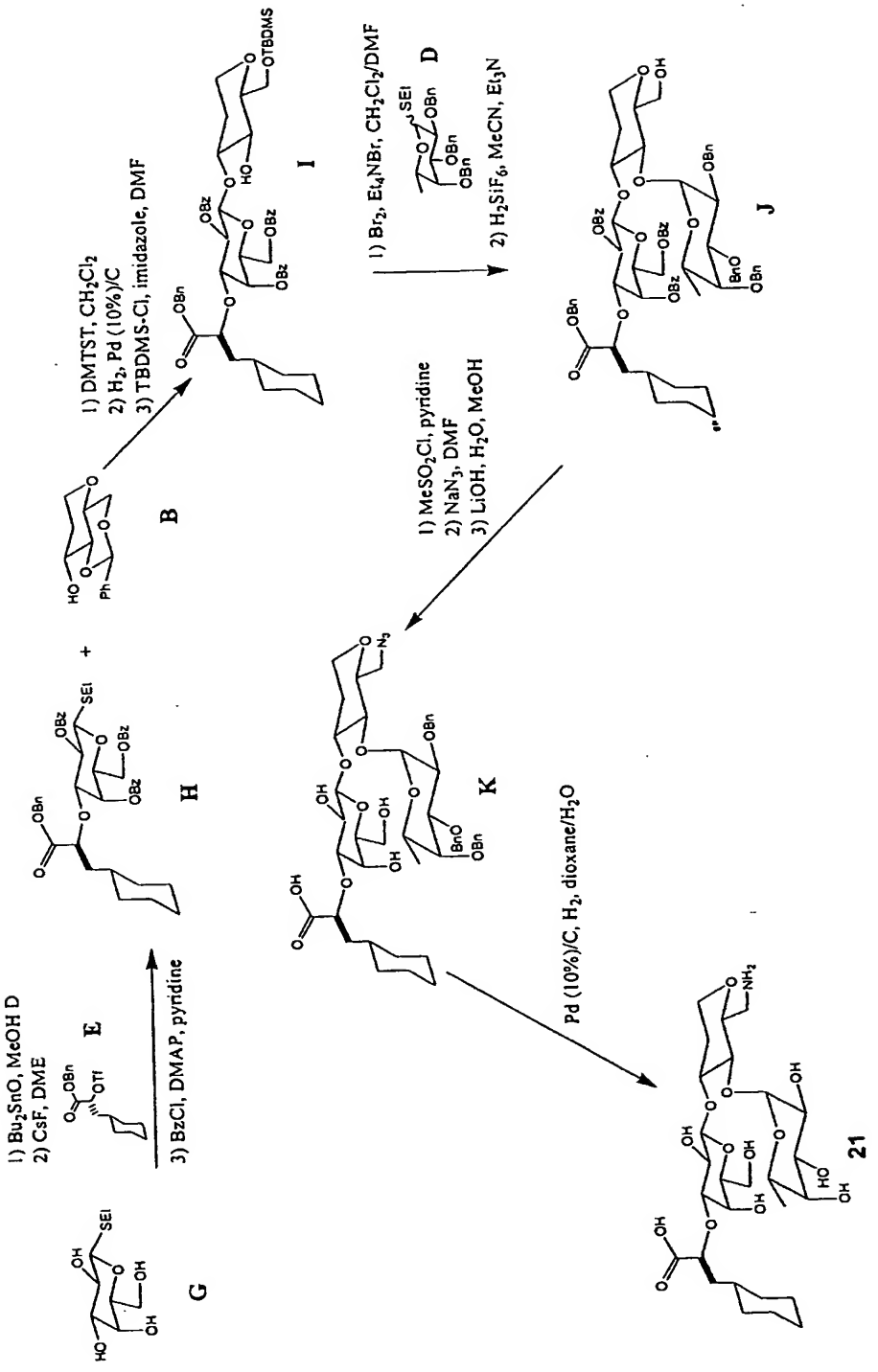

1) Bu_2SnO, MeOH D
2) CsF, DME
3) BzCl, DMAP, pyridine
1) DMTST, CH_2Cl_2
2) H_2, Pd (10%)/C
3) TBDMS-Cl, imidazole, DMF
1) Br_2, Et_4NBr, CH_2Cl_2/DMF
2) H_2SiF_6, MeCN, Et_3N
1) $MeSO_2Cl$, pyridine
2) NaN_3, DMF
3) LiOH, H_2O, MeOH
Pd (10%)/C, H_2, dioxane/H_2O
G
E
B
I
H
D
J
K
21

FIG. 4

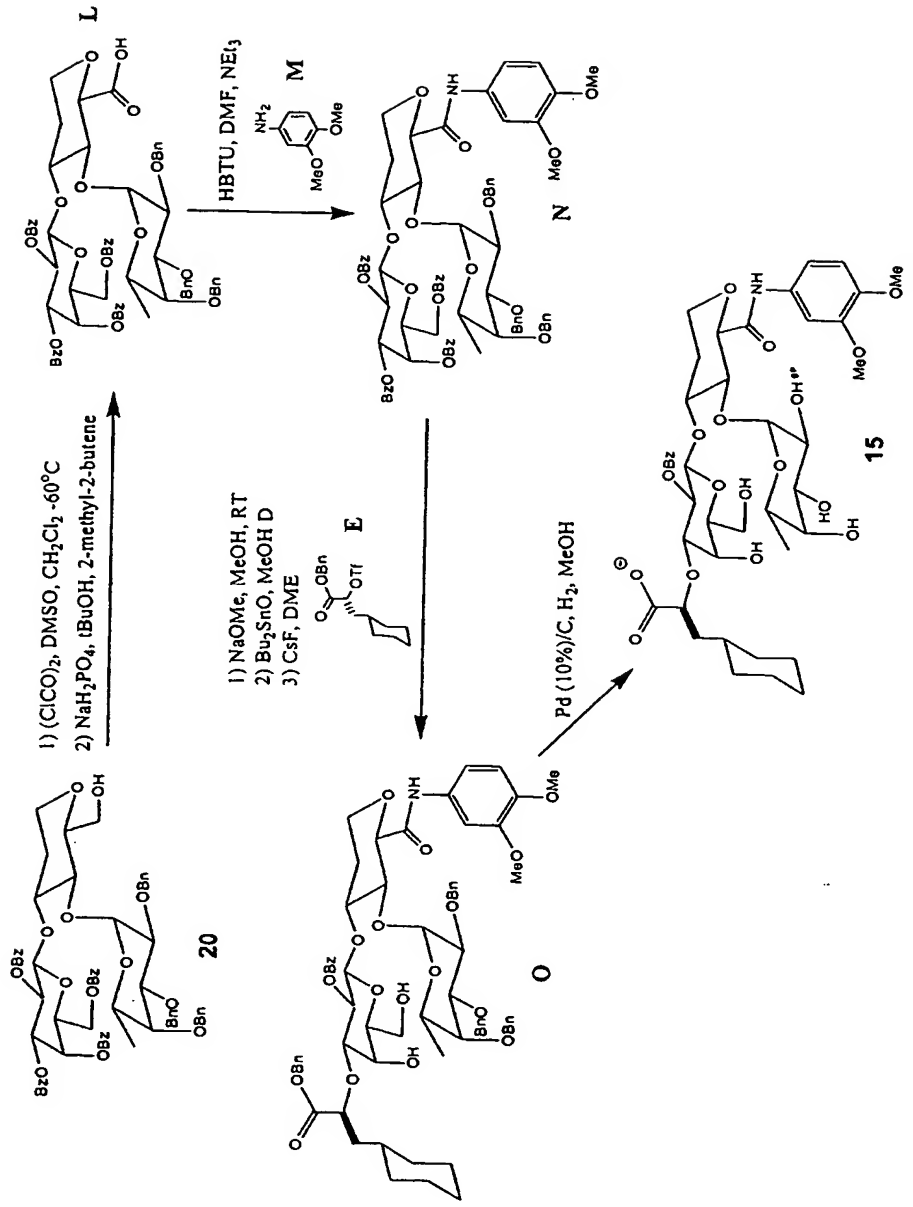

L
M
N
O
20
15
E
1) (ClCO)2, DMSO, CH2Cl2 -60°C
2) NaH2PO4, tBuOH, 2-methyl-2-butene
HBTU, DMF, NEt3
1) NaOMe, MeOH, RT
2) Bu2SnO, MeOH D
3) CsF, DME
Pd (10%)/C, H2, MeOH

FIG. 5

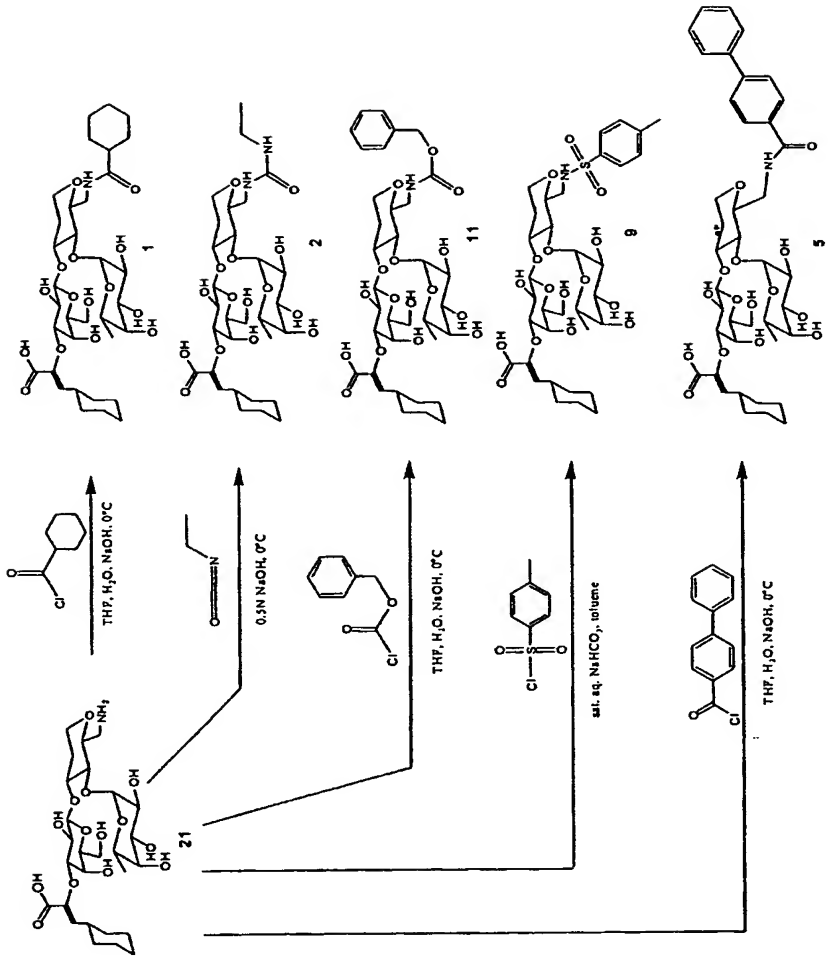

THF, H2O, NaOH, 0°C
0.3N NaOH, 0°C
THF, H2O, NaOH, 0°C
sat. aq. NaHCO3, toluene
THF, H2O, NaOH, 0°C
21
1
2
11
8
5

FIG. 6

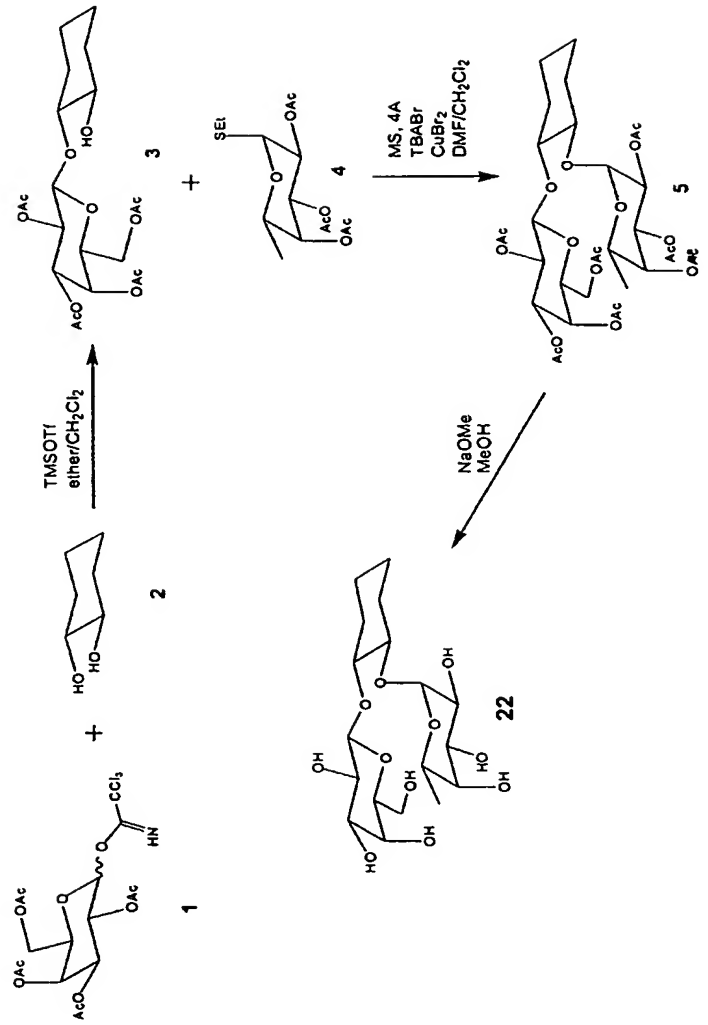

TMSOT/
ether/CH2Cl2
MS, 4A
TBABr
CuBr2
DMF/CH2Cl2
NaOMe
MeOH
1
2
3
4
5
22

FIG. 7

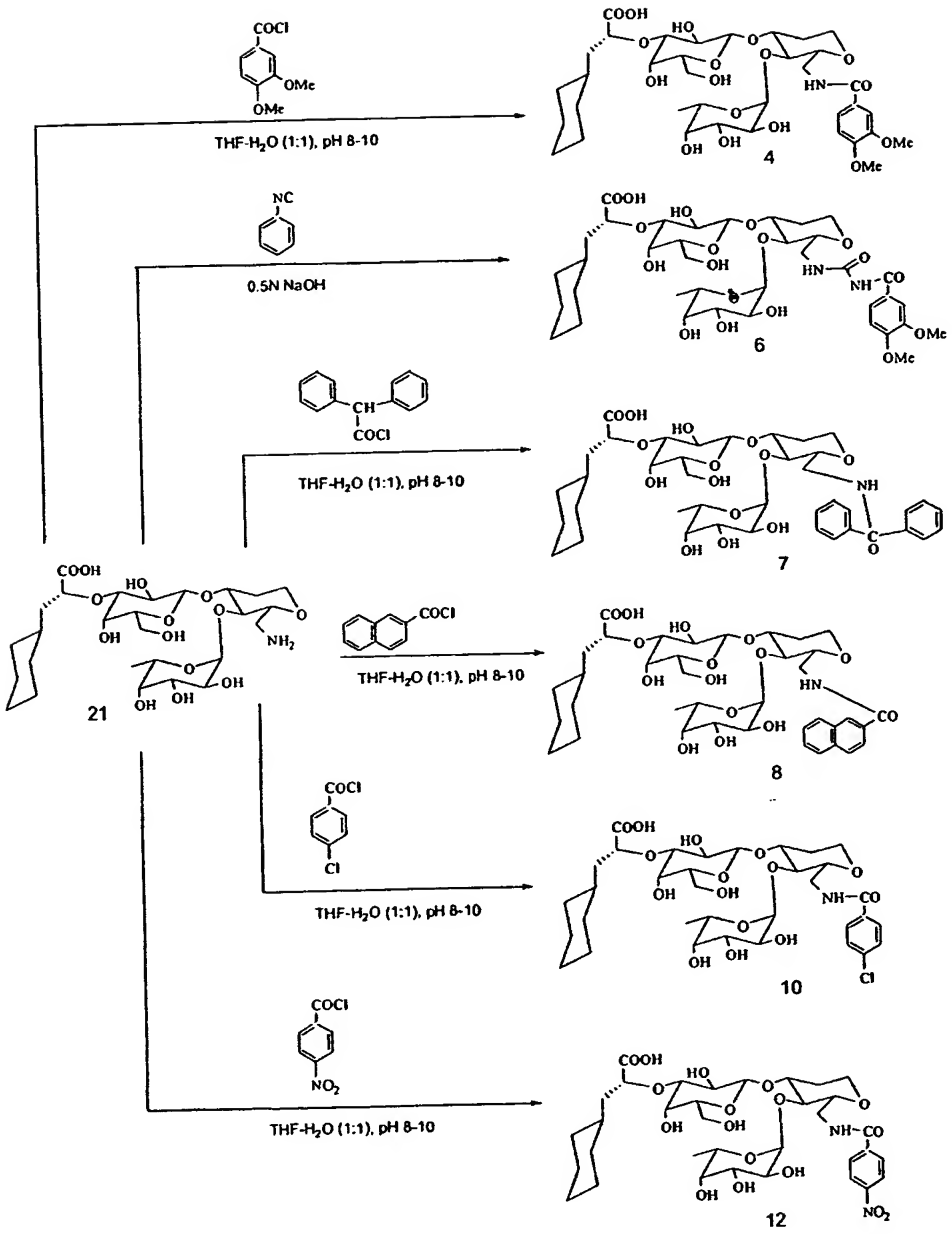

COCl
OMe
OMe
THF-H2O (1:1), pH 8-10
4
NC
0.5N NaOH
6
CH
COCl
THF-H2O (1:1), pH 8-10
7
21
COCl
THF-H2O (1:1), pH 8-10
8
COCl
Cl
THF-H2O (1:1), pH 8-10
10
COCl
NO2
THF-H2O (1:1), pH 8-10
12

FIG. 8A

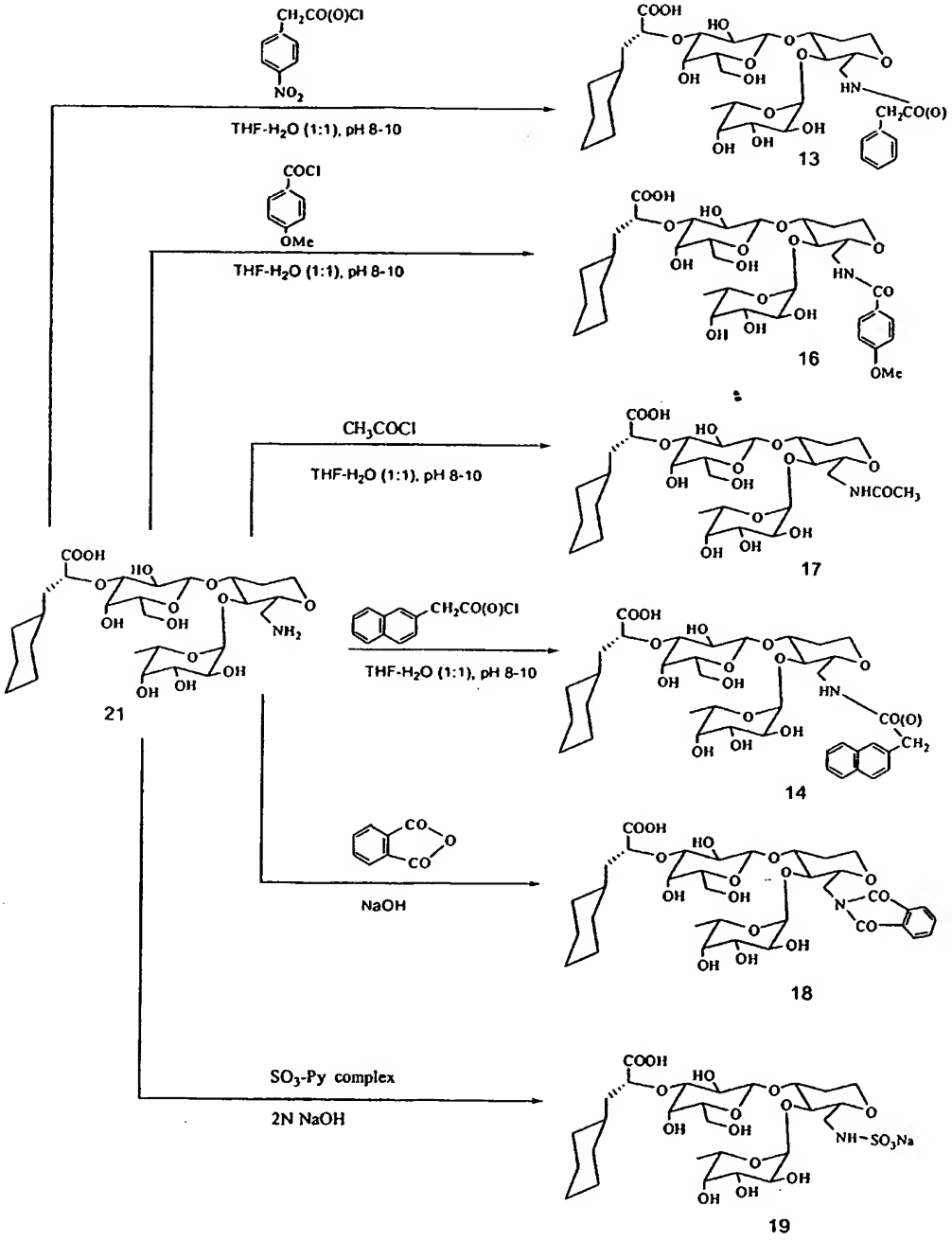

CH2CO(O)Cl
NO2
THF-H2O (1:1), pH 8-10
13
COCl
OMe
THF-H2O (1:1), pH 8-10
16
OMe
CH3COCl
THF-H2O (1:1), pH 8-10
17
21
CH2CO(O)Cl
THF-H2O (1:1), pH 8-10
14
NaOH
18
SO3-Py complex
2N NaOH
19

FIG. 8B

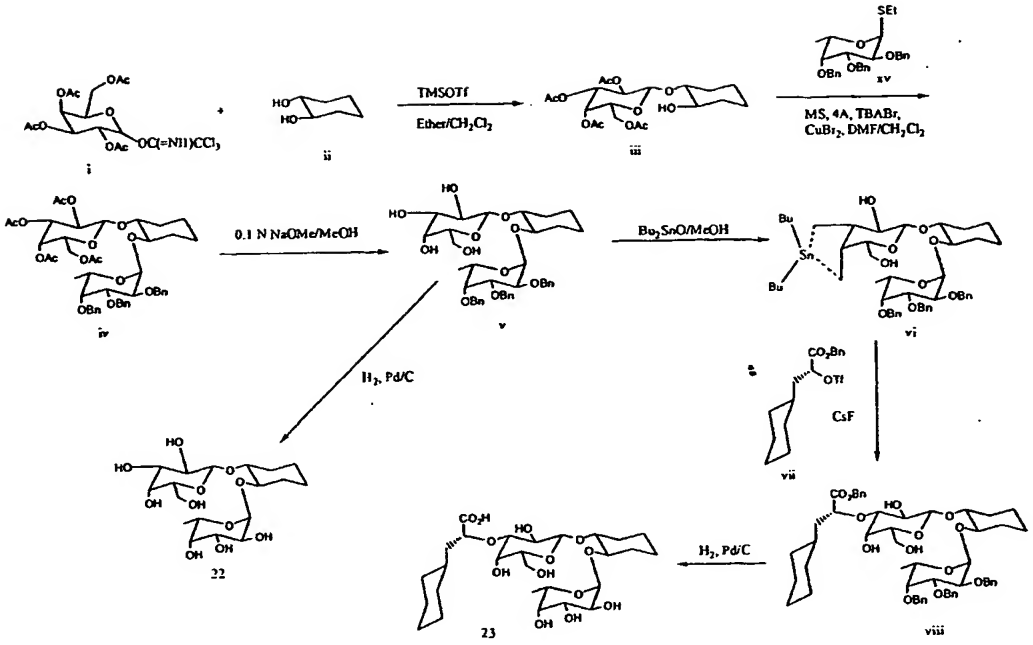

TMSOTf
Ether/CH2Cl2
MS, 4A, TBABr,
CuBr2, DMF/CH2Cl2
0.1 N NaOMe/MeOH
Bu2SnO/MeOH
H2, Pd/C
CsF
H2, Pd/C
22
23

FIG. 9

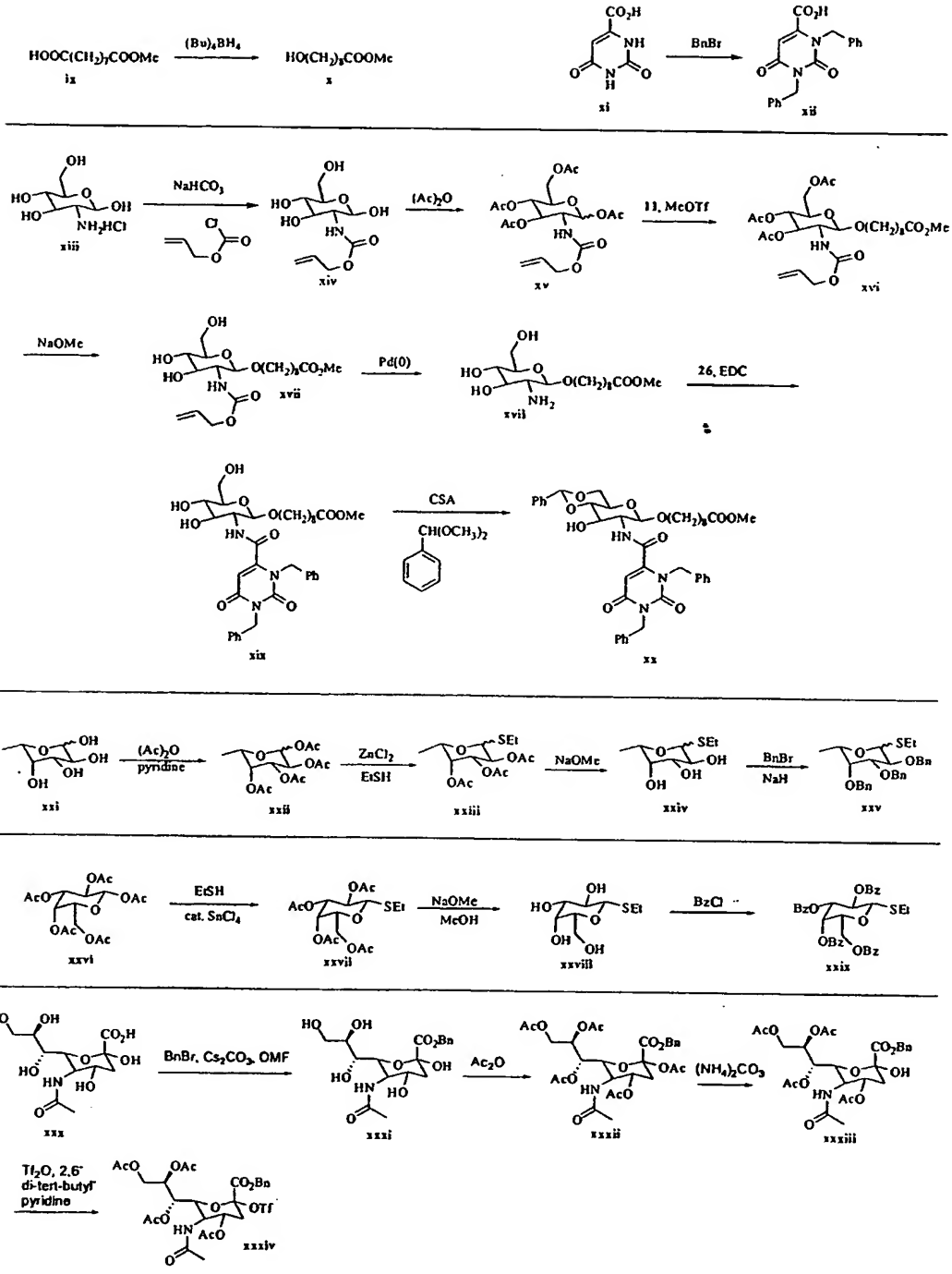


FIG. 10

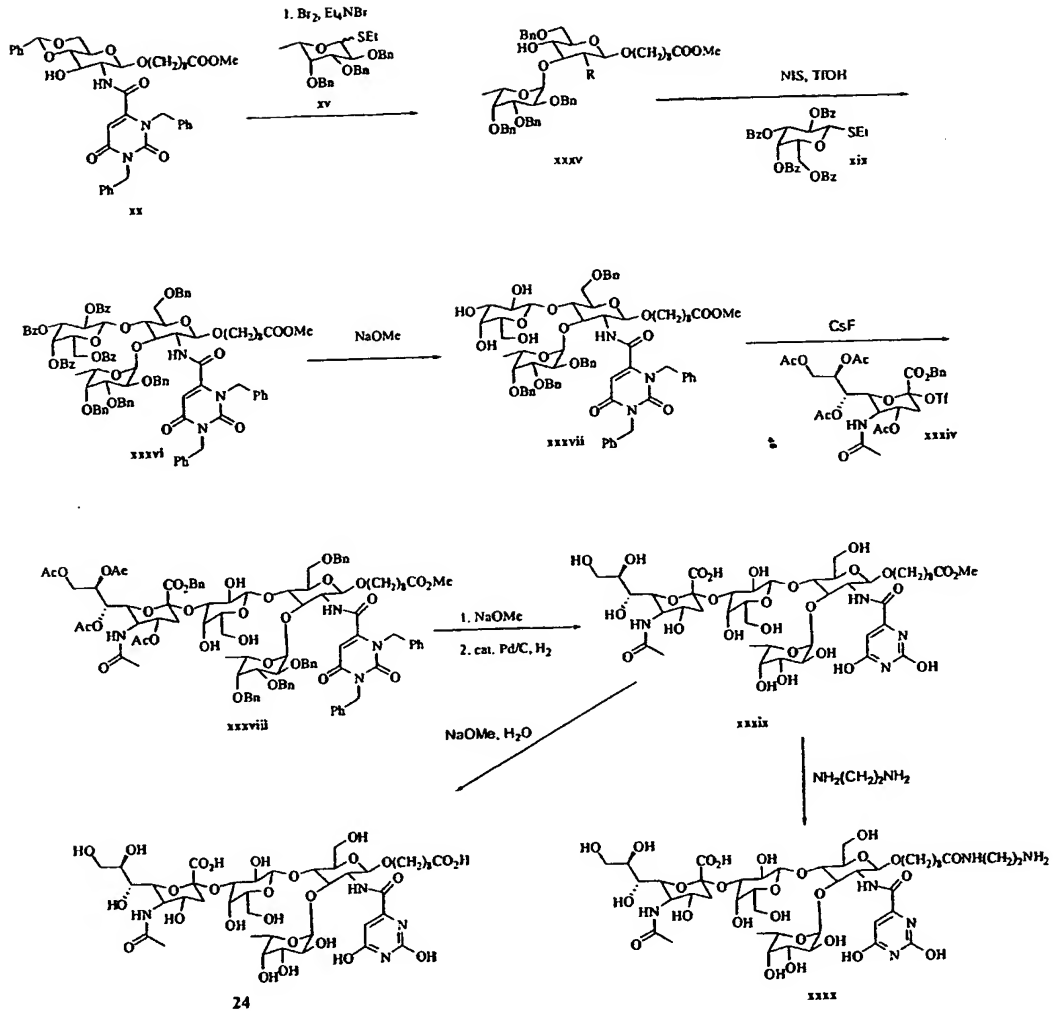

1. Br2, Et4NBr
NIS, TfOH
NaOMe
CsF
1. NaOMe
2. cat. Pd/C, H2
NaOMe, H2O
NH2(CH2)2NH2
xx
xv
xxxv
xix
xxxvi
xxxvii
xxxiv
xxxviii
xxxix
24
xxxx

FIG. 11

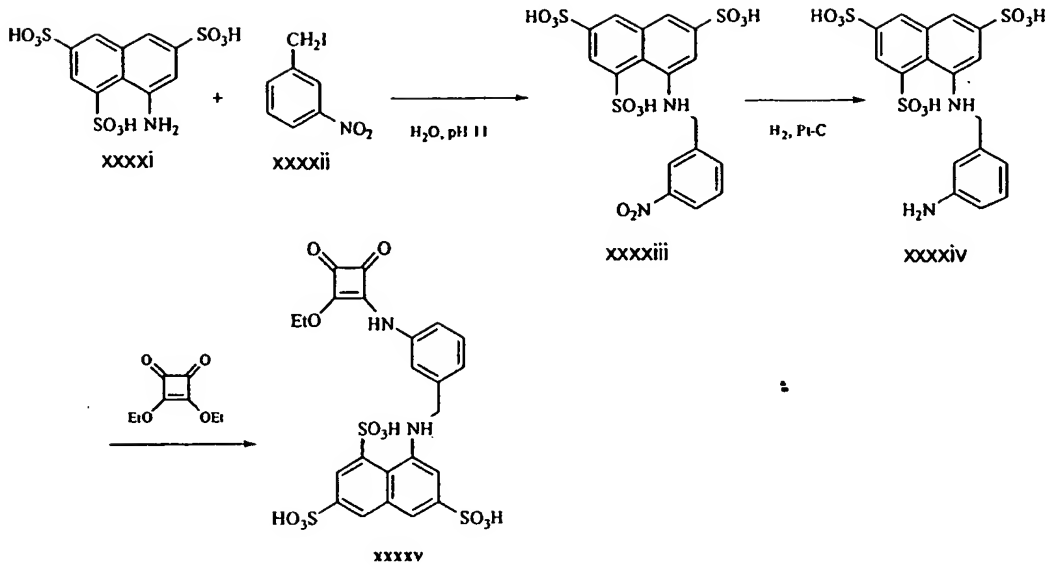

HO3S
SO3H
SO3H NH2
xxxxi
CH2I
NO2
xxxxii
H2O, pH 11
SO3H NH
O2N
xxxxiii
H2, Pt-C
H2N
xxxxiv
EtO
OEt
HN
xxxxv

FIG. 12

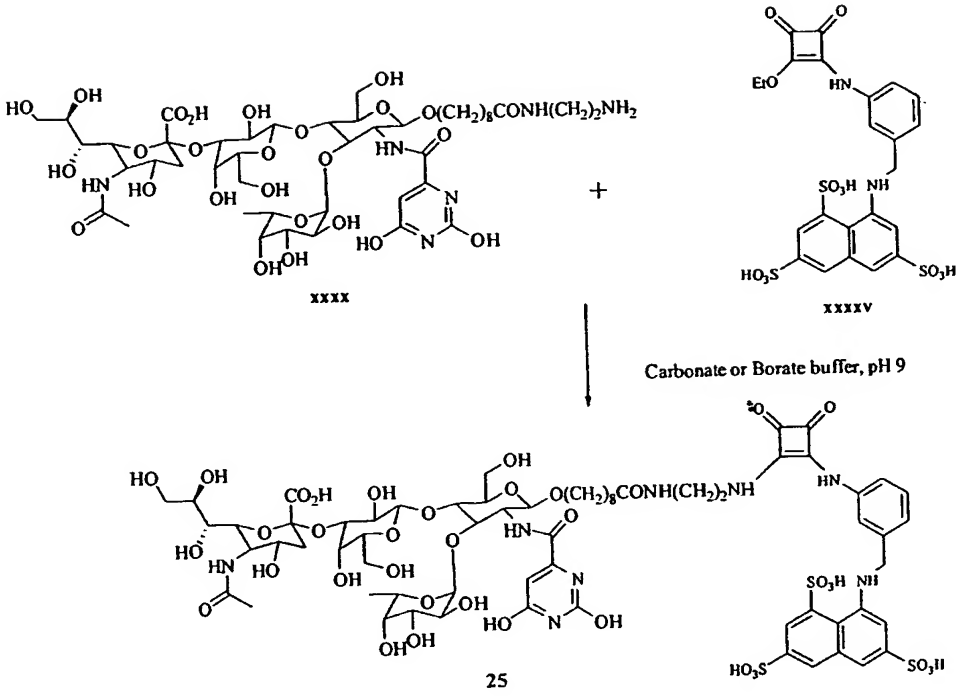


FIG. 13

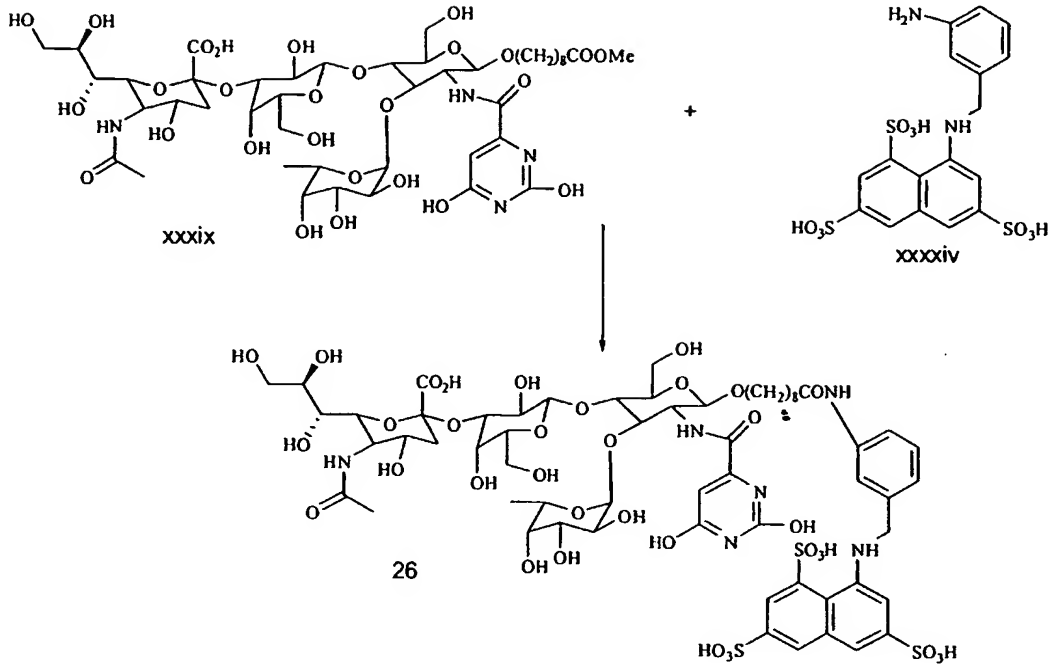


FIG. 14

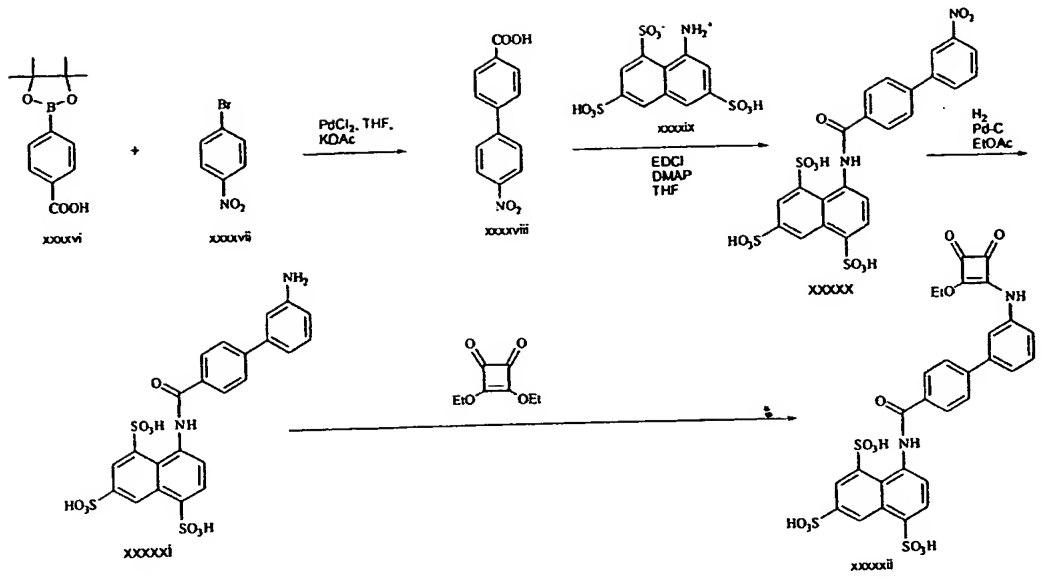

PdCl2, THF, KOAc
EDCI DMAP THF
H2 Pd-C EtOAc
xxxxvi
xxxxvii
xxxxviii
xxxxix
xxxxx
xxxxxi
xxxxxii

FIG. 15

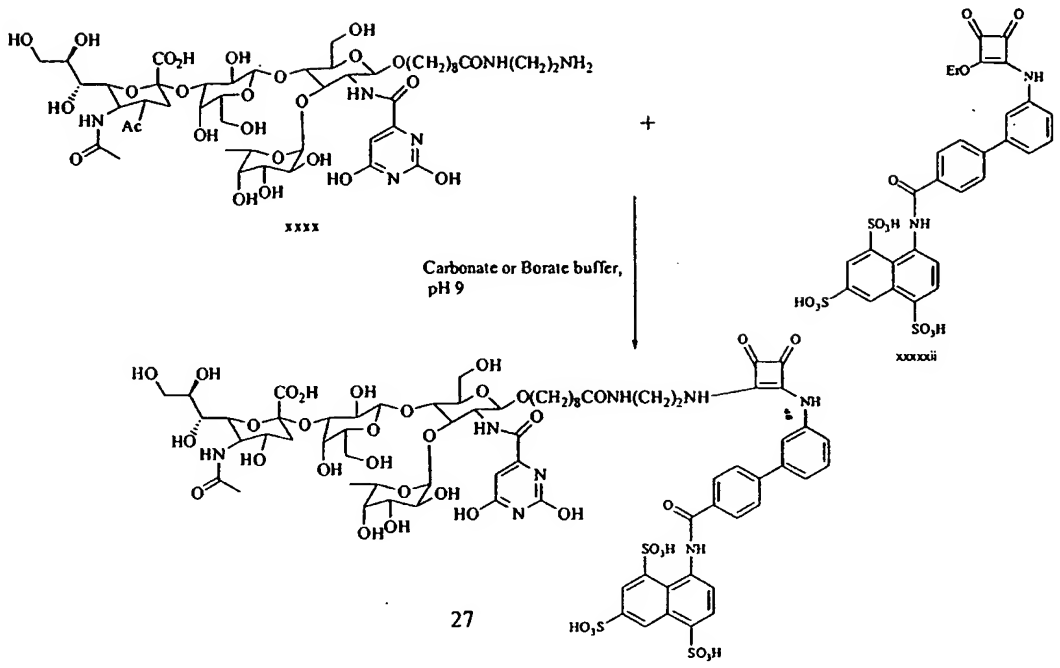


FIG. 16

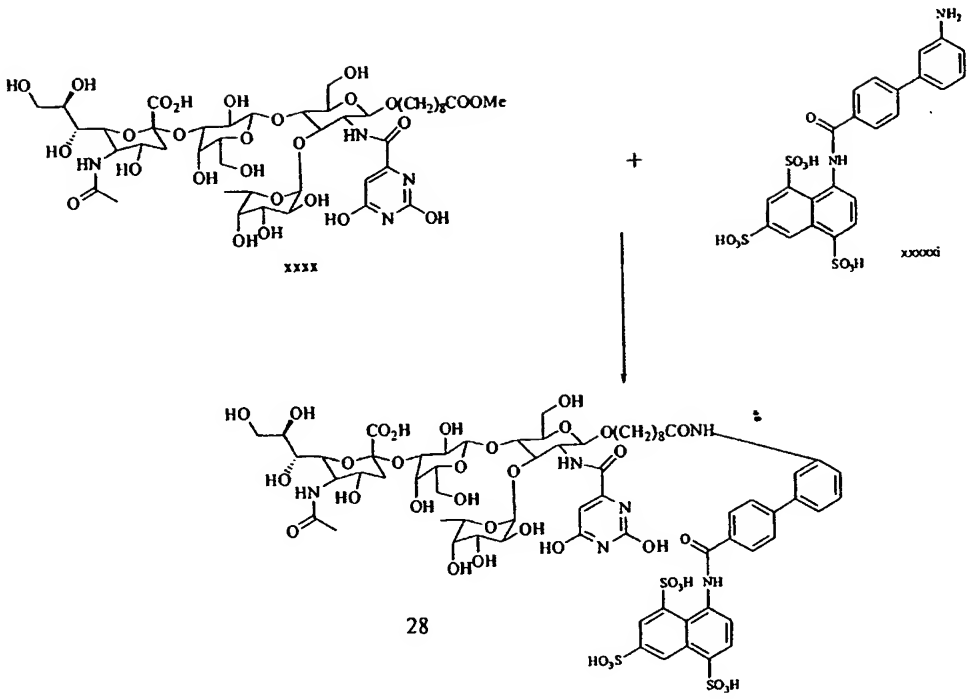


FIG. 17

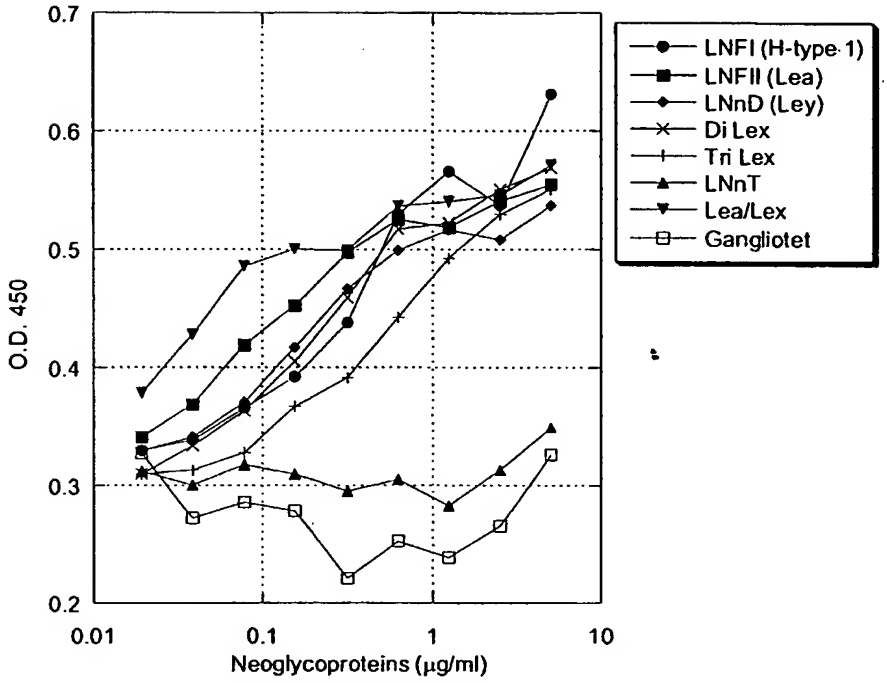

0.7
0.6
0.5
0.4
0.3
0.2
O.D. 450
0.01
0.1
1
10
Neoglycoproteins (µg/ml)
LNFI (H-type 1)
LNFII (Lea)
LNnD (Ley)
Di Lex
Tri Lex
LNnT
Lea/Lex
Gangliotet

FIG. 18

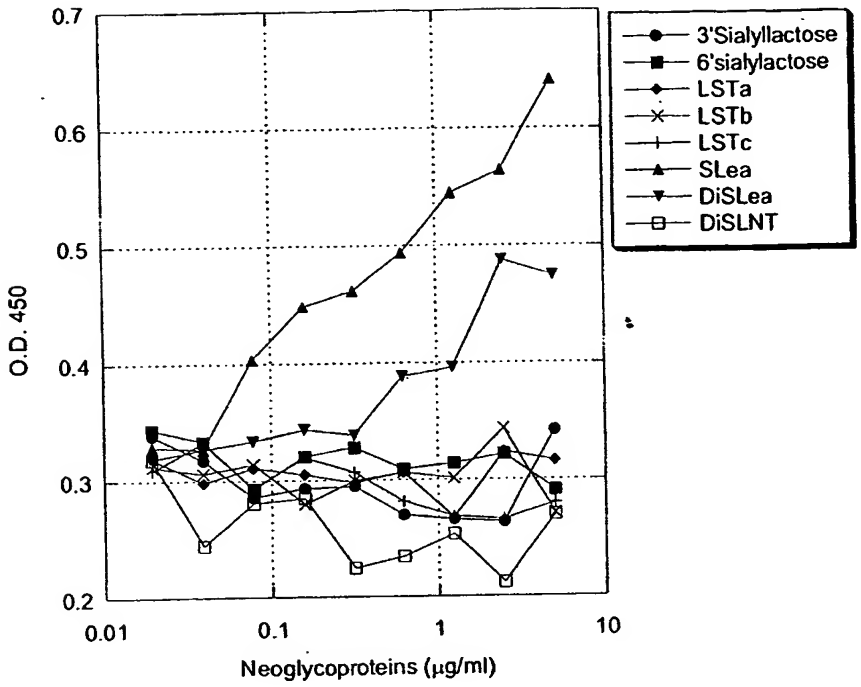

0.7
0.6
0.5
0.4
0.3
0.2
O.D. 450
0.01
0.1
1
10
Neoglycoproteins (µg/ml)
3'Sialyllactose
6'sialylactose
LSTa
LSTb
LSTc
SLea
DiSLea
DiSLNT

FIG. 19

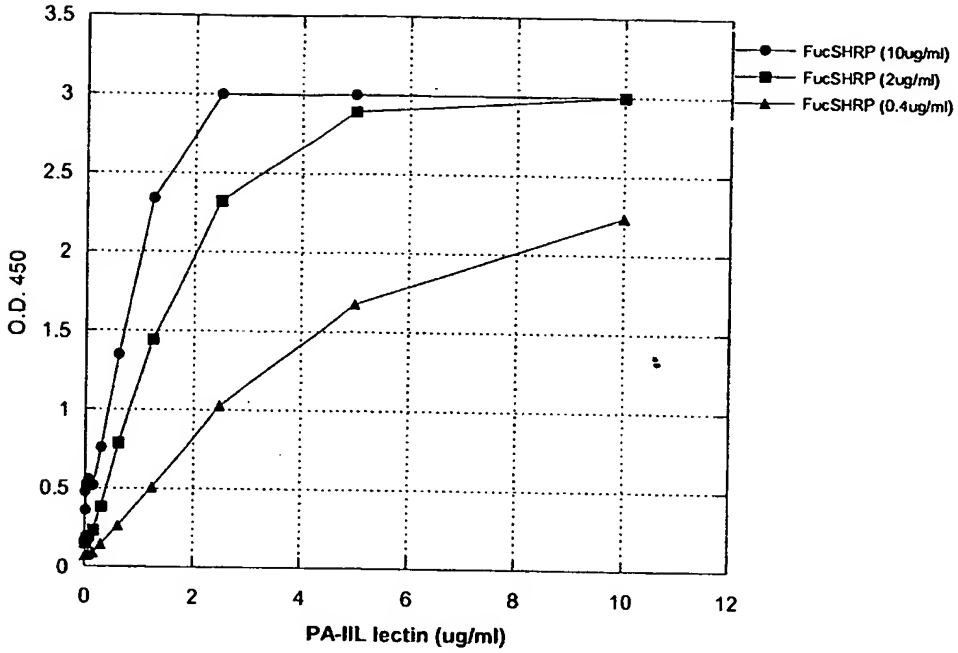

3.5
3
2.5
2
1.5
1
0.5
0
O.D. 450
0
2
4
6
8
10
12
PA-IIL lectin (ug/ml)
FucSHRP (10ug/ml)
FucSHRP (2ug/ml)
FucSHRP (0.4ug/ml)

FIG. 20

Inhibition Assay (IC_{50}) for PA-IIL Lectin

Microtiter-based one step assay for high throughput

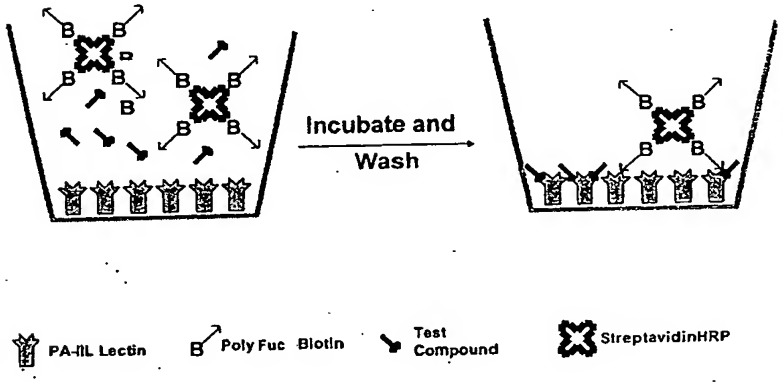


FIG. 21

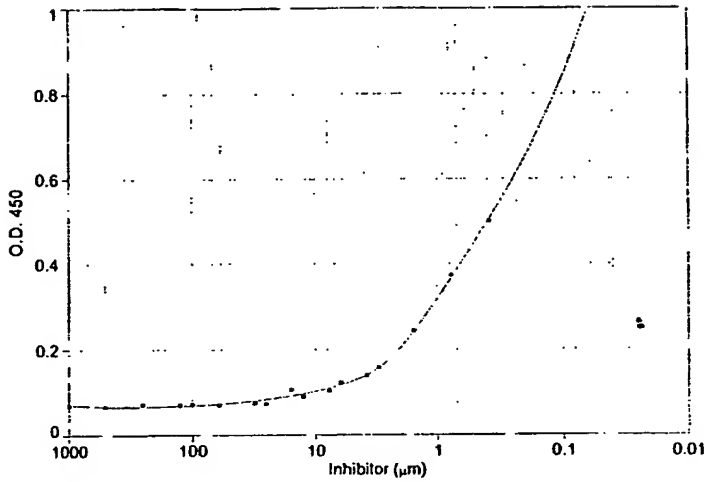


FIG. 22

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/40881

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48 A61K31/702

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/42130 A (CONNAUGHT LAB ;CHONG PELE (CA); KLEIN MICHEL H (CA); LINDBERG ALF) 26 August 1999 (1999-08-26) page 29, line 22 -page 30, line 7; claim 14 ---	1-6, 10-17
X	WO 97/28173 A (CIBA GEIGY AG ;OEHRLEIN REINHOLD (DE)) 7 August 1997 (1997-08-07) see compounds 22, 26 page 41; claim 44; examples B4.1,,B4.2 ---	5,6, 10-14
X	WO 97/28174 A (CIBA GEIGY AG ;OEHRLEIN REINHOLD (DE)) 7 August 1997 (1997-08-07) page 16; claims 47,48; example B2.5 --- -/--	5,6, 10-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April 2004	26/04/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gonzalez Ramon, N

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHARFMAN A. ET AL: "Recognition of Lewis X derivatives present on mucins by flagellar components of Pseudomonas aeruginosa" INFECTION AND IMMUNITY, vol. 69, no. 9, September 2001 (2001-09), pages 5243-5248, XP001187580 page 5244, column 1, paragraph 5 abstract; table 1 ---	7-9
Y	SCHARFMAN ANDREE ET AL: "Pseudomonas aeruginosa binds to neoglycoconjugates bearing mucin carbohydrate determinants and predominantly to sialyl-Lewis x conjugates" GLYCOBIOLOGY, vol. 9, no. 8, August 1999 (1999-08), pages 757-764, XP008029519 ISSN: 0959-6658 abstract; table 1 page 762, column 1 ---	1-17
Y	BÄNTELI R ET AL: "Potent E-Selectin Antagonists" HELVETICA CHIMICA ACTA, VERLAG HELVETICA CHIMICA ACTA. BASEL, CH, vol. 83, 2000, pages 2893-2907, XP002257742 ISSN: 0018-019X	1-17
X	see compounds 2, 5 page 2897; table 1 ---	10-12
Y	THOMA G ET AL: "A Readily Available, Highly Potent E-Selectin Antagonist" ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, VERLAG CHEMIE. WEINHEIM, DE, vol. 40, no. 19, 2001, pages 3644-3647, XP002257743 ISSN: 0570-0833	1-17
X	see compound 4 abstract; table 1 ---	10-12
A	SPRENGARD U ET AL: "Synthesis and Biological Activity of Novel Sialyl-LewisConjugates" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 6, no. 5, 5 March 1996 (1996-03-05), pages 509-514, XP004135018 ISSN: 0960-894X abstract; figure 1 --- -/--	1-17

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/40881

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/43356 A (HSC RES DEV LP) 2 September 1999 (1999-09-02) page 17, line 35; claims 1,9,17,22,30,37,61,62; table 1	1-17
P,X	WO 03/097658 A (GLYCOTECH INC ;PATTON JOHN T (US); MAGNANI JOHN L (US); WILLIAMS L) 27 November 2003 (2003-11-27) page 10, line 16 - line 35; claims 1-5 page 12, line 18 - line 19	5,6, 10-14
P,X	MAGNANI J L: "POTENT GLYCOMIMETIC INHIBITORS OF THE ADHESION MOLECULE, PA-IIL, FOR THE BACTERIAL PATHOGEN, PSEUDOMONAS AERUGINOSA" GLYCOBIOLOGY, IRL PRESS,, GB, vol. 13, no. 11, October 2003 (2003-10), page 854 XP008029473 ISSN: 0959-6658 abstract	1-6, 10-17

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-4 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claims 7,8 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: partially 1-17

Definition of a compound by reference to a figure (claims 1, 5, 7, 9, 10, 15) is unclear (Art. 5. PCT).

Present claims 2,3, 5-8, 13, 14, 16, 17 relate to an extremely large number of possible compounds defined as "therapeutic agent" (claims 2, 5); "diagnostic agent" (claim 7).
Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed.
In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.
Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds prepared in the examples and those compounds specifically defined by chemical name in the description at pages 6/7.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/40881

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: partially 1-17
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 03/40881

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9942130	A	26-08-1999	US	2001048929	A1	06-12-2001
			AU	754021	B2	31-10-2002
			AU	2606499	A	06-09-1999
			BR	9908163	A	06-11-2001
			CA	2321973	A1	26-08-1999
			WO	9942130	A1	26-08-1999
			EP	1056470	A1	06-12-2000
			JP	2002503705	T	05-02-2002
			NZ	506617	A	25-07-2003
			US	6656472	B1	02-12-2003
WO 9728173	A	07-08-1997	AU	1542397	A	22-08-1997
			WO	9728173	A1	07-08-1997
			EP	0886640	A1	30-12-1998
			US	6169077	B1	02-01-2001
WO 9728174	A	07-08-1997	AU	1444697	A	22-08-1997
			WO	9728174	A1	07-08-1997
			EP	0886639	A1	30-12-1998
			US	6187754	B1	13-02-2001
WO 9943356	A	02-09-1999	AU	6084598	A	18-09-1998
			AU	8967998	A	15-09-1999
			WO	9943356	A1	02-09-1999
WO 03097658	A	27-11-2003	WO	03097658	A2	27-11-2003